PROCEEDINGS

OF THE

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

VOL. 30. MARCH, 1933. NO. 6.

Illinois Section.

University of Illinois College of Medicine, February 14, 1933.

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Temperature Changes in the Liver in Relation to Heat and Cold on Skin.

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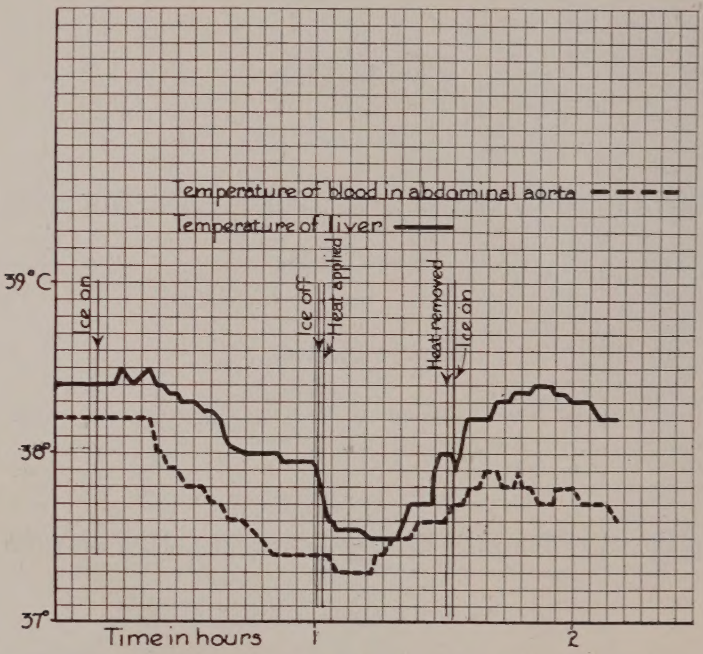
For recording the temperature in the liver we used a fever recorder of Siemens and Halske. To this apparatus were devised special electrodes of a size suitable to requirements of the case, made of a piece of flat mica wound around by an iron wire of a diameter of 0.00175 in. The electrodes were insulated on one side by cork and mica and on the other side (sensitive part) with shellac and celluloid. Both ends of a wire were attached to heavier wires, connected with the galvanometer and the latter with the fever recorder.

Six dogs were used. Under nembutal anesthesia, the abdomen was opened and the flat electrode placed between the lobes of the liver, fastened by its wires to the external surface of the abdominal wall by means of an adhesive plaster and the abdomen closed. Heparin was injected intravenously (0.01 per kilo), the femoral artery opened and a long glass thermometer introduced through it into the abdominal aorta. Ice packs and hot moist towels were applied to the skin of the chest of dogs.



The results are shown on an accompanying chart. The abscissa represents the time from the beginning of the experiment, the ordinate—temperature in Centigrade. Dotted lines represent the findings of the temperature of the blood in the abdominal aorta, the straight line the temperature of a surface of one of the lobes of the liver. The perpendicular lines show the time of application of cold and heat to the body surface of the dog.

The temperature of the liver and abdominal aorta show that the liver tends to maintain its temperature in comparison with that of the blood. The influence of heating and cooling the skin upon the systemic blood can be observed. The liver has a tendency to maintain its temperature at a higher level than the blood.

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Temperature Changes in Trachea and Kidney in Relation to Heat and Cold on Skin.

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From the Department of Bacteriology and Preventive Medicine, University of Illinois, College of Medicine, and Research Laboratories of the Illinois Department of Public Health.

The technic in these experiments was exactly the same as described in the preceding article. The only difference was the electrodes, which, though of the same device, construction and shape, were of different size and applied to different objects. Here we present our findings of the temperature of the mucous membrane of the trachea and normal and denervated kidneys.

In the case of measuring the temperature of the mucous membrane of the trachea, dogs were used. The tracheotomy was performed under nembutal anesthesia, the electrode applied flatly to the surface of the mucous membrane of the trachea, fastened by a piece of cork, the latter resting on the opposite side of the trachea. Then the incision was closed.

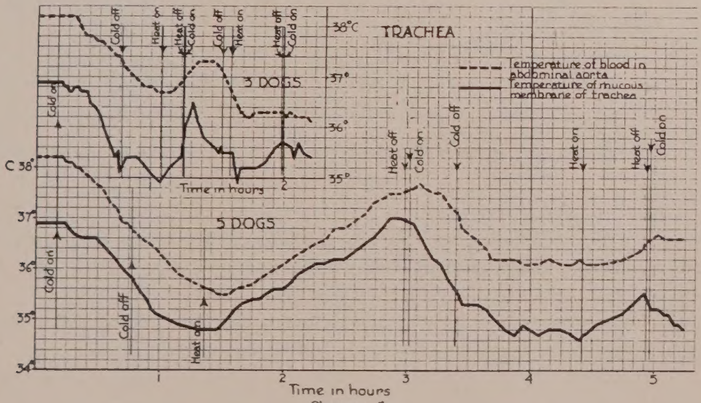

Chart 1.

On the first chart are all the average readings of the temperature of the mucous membrane of 8 dogs, which we divided in two groups. The upper curves on the chart represent 3 dogs and the lower 5. The reason for this is that the dogs reacted quite differently: the smaller group were more sensitive, the larger more stable. But the general conclusions are that the trachea responds to the exposure of the skin to cold and heat the same as the skin itself.

For measuring the temperature of the kidney's surface we exposed the organ, placed the electrode flatly to the kidney, fastened it with adhesive plaster, replaced the kidney and closed the wound.

The same can be said concerning the normal kidney (3 dogs) which follows in general the reaction of the trachea. In the denervated kidney (2 dogs) there is a great difference in comparison with the normal; the temperature of a surface of the kidney follows quite closely the temperature of the blood. (Chart 2.)

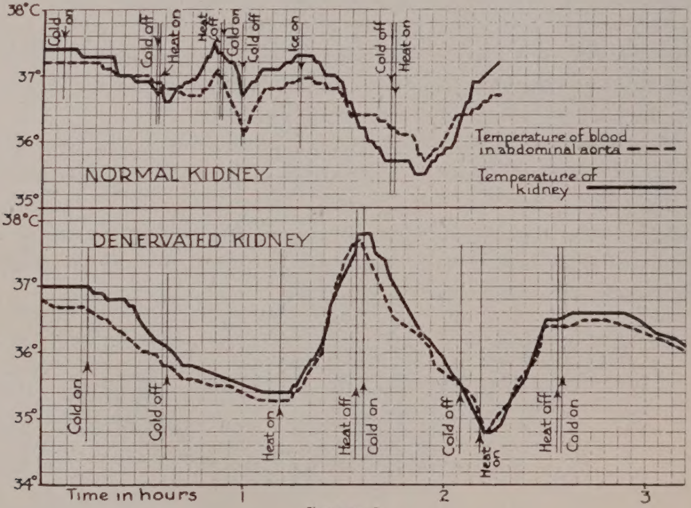

CHART 2.

These experiments could be done only because of special electrodes devised by Dr. A. Bachem of this college and because of his constant assistance. It is a pleasure to acknowledge our indebtedness to him.

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Delayed Sugar Utilization by Bacteria.

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From the Department of Bacteriology, Northwestern University Medical School.

From one of the variants (C16) obtained by Nungester and Anderson[1] in a study of a recently isolated *B. coli*-like organism, Nungester[2] has in later work obtained 7 additional variants. These agglutinate in similar high dilutions of the parent immune serum but differ from each other in their reactions on lactose, maltose and sucrose Andrade agar as indicated in the table.

TABLE I.
Carbohydrate Reactions of C16 and its Variants.

Variant	Source	Lactose	Maltose	Sucrose
C16	—	—	—	—
C17	C16	+	—	—
C19	C16	—	+	—
C20	C16	—	—	+
C18	C17	+	+	—
C21	C19	—	+	+
C23	C20	+	—	+
C22	C18	+	+	+

In carbohydrate broth medium 2 types of reactions occurred: (1) in broths containing the same kind of sugars as in the indicator plates which gave positive reactions, acid was produced in one day; (2) in broths containing the same kind of sugars as in the indicator plates which gave negative reactions, acid production was delayed and appeared after one to 10 days.

Jones, Orcutt and Little[3] have reported atypical slow lactose fermenting colon bacilli which appear somewhat similar to those described above. Alkalinity was maintained in broth for several days before acid was evident. In all cases, however, quantitative analysis showed that lactose was being consumed from the first day of growth. The authors postulate that although lactose is used from the start, sufficient alkali is produced to mask the fermentation.

It therefore seemed advisable to determine whether the same mechanism was operative in the case of C16 and its variants or whether this delay in acid production corresponded to a postponement of sugar utilization. For this purpose, two basic mediums were used: (1) sugar-free beef infusion broth and (2) 2% peptone 0.5% sodium chloride solution. To each was added 10% lactose or maltose solution, sterilized by filtration, to make a final concentration of about 0.8%. These mediums were then tubed (10-15 cc. per tube), incubated several days for sterility, and inoculated either from agar slants or from 24-hour carbohydrate indicator agar plate cultures. The daily hydrogen ion concentrations were taken by means of a colorimetric La Motte Hydrogen Ion Testing Set. Quantitative sugar determinations were made daily or every other day by the Stiles, Peterson, and Fred[4] copper reduction method. Two or more tubes were used for each organism and determinations were repeated several times with different batches of medium.

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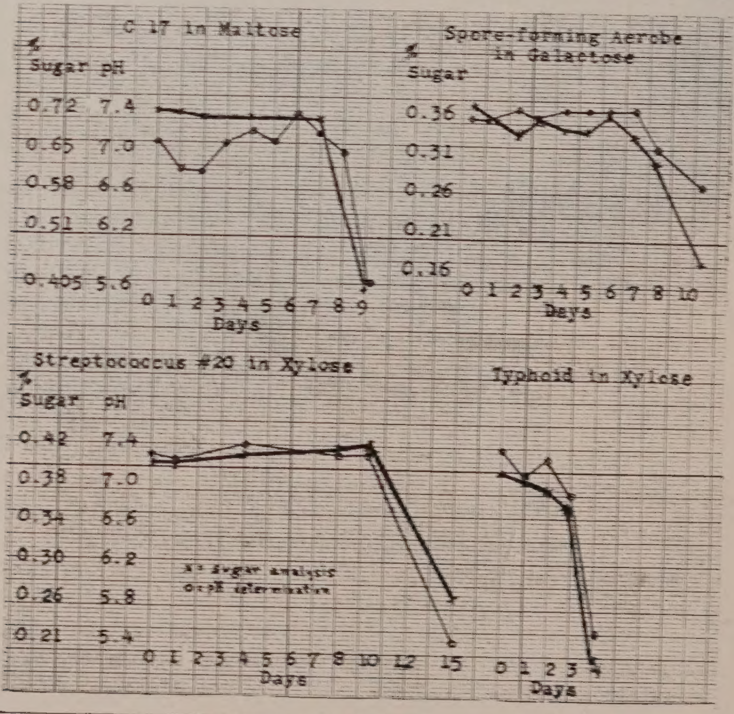
[1] Nungester, W. J., and Anderson, S. A., *J. Inf. Dis.*, 1931, **49**, 455.

[2] Nungester, W. J., *J. Bact.*, 1933, **25**, 49.

[3] Jones, F. S., Orcutt, M., and Little, B. B., *J. Bact.*, 1932, **23**, 267.

C16, 19, 20, and 21 in lactose, and C16, 17, 20, and 23 in maltose all showed delayed sugar utilization and a correspondingly delayed development of acidity. The reaction would remain neutral or become slightly alkaline; then over night there would be a shift to the



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[4] Stiles, H. R., Peterson, W. H., and Fred, E. B., *J. Bact.*, 1926, **12**, 427.

acid side accompanying a sudden use of the sugar. The length of the latent period in a given medium varied with the different variants from one to 10 days but was fairly constant with each variant. The graph for C17 records the hydrogen ion concentration and the percentage of sugar at different incubation intervals and is representative of the curves obtained for all the variants.

The graph shows that for a number of days no sugar was utilized and that sudden utilization of sugar was accompanied by a sudden shift to acidity. When such tubes were plated out, the cultures were found to be without contamination. Also subcultures from the acid broths (in which sugar had been utilized) to the appropriate carbohydrate indicator agar plates often failed to show any acid production. This is in agreement with Nungester's studies on these organisms which will be reported at a later date.

In addition to C16 and its variants, 2 other coli-like organisms, freshly isolated, gave the same type of delayed reactions in lactose, although they produced acid in maltose and sucrose in one day. From these 2 organisms could be developed variants which produced acid in lactose broth in one day.

In the course of quantitative determinations of sugar use by various bacteria, 3 other microorganisms were found to give this same type of delayed utilization. The Rawlins strain of *B. typhosus* carried for a number of years in the laboratory produced, in xylose peptone medium, curves of pH and sugar use similar to those of C16. A streptococcus did the same. A spore-forming aerobe was found to act similarly in galactose media. The graphs are illustrative.

It therefore seems that certain organisms are capable of growing in carbohydrate liquid media for several days without using any of the sugar. When suddenly sugar begins to be used, there is a corresponding increase in acidity. These observations do not necessarily question those of Jones, Orcutt and Little, for there are at hand in this and in other laboratories several instances of slow sugar used by bacteria either without appreciable alteration of pH or with increasing alkalinity.

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Disappearance of Vitamin C from Adrenals of Guinea Pigs Having Scurvy.

A. E. SIEHRS AND C. O. MILLER. (Introduced by C. J. Farmer.)

From the Northwestern University Medical School.

Szent-Gyorgyi[1] reported that the cortex of sliced beef adrenals turn dark when placed in a neutral 0.4% solution of silver nitrate and that the reducing substance is an hexuronic acid. King and Waugh[2] reported that the crystalline vitamin C which they isolated from lemon juice is identical with the hexuronic acid from adrenals obtained by Szent-Gyorgyi. We reported[3] that the cortex and medulla of sliced adrenals of normal guinea pigs turn dark in a neutral 0.4% solution of silver nitrate, but that the cortex from guinea pigs having scurvy does not darken under similar conditions. This confirms the conclusions of King and Waugh. We had observed the action of the adrenals in 6 normal guinea pigs and 6 with scurvy. We have increased this number to 42. We have investigated also the time required for the disappearance of vitamin C from the adrenals of guinea pigs on a scorbutic diet and the time for its reappearance in the adrenals when orange juice is added to the scorbutic diet. Recently Moore and Ray[4] have also reported that adrenals from guinea pigs having scurvy do not reduce neutral 0.4% silver nitrate, which confirms our first observation.

Twenty-five guinea pigs, weighing 150-180 gm., were fed oats and carrots for 8 days. On the eighth day, 2 were killed and the reducing power of their adrenals was noted. The remaining animals were fed the scorbutic diet of Sherman, LaMer, and Campbell.[5] Ten animals were killed at various intervals from 2 to 18 days of the diet period. The adrenals were removed, one from each animal was sliced and placed in a neutral 0.4% silver nitrate solution for 15 minutes. The pieces were exposed to the light from a 115 watt Mazda bulb at a distance of 8 inches. Both the cortex and the medulla reduced the silver nitrate; however, the medulla reduces more slowly than the cortex. The cut surfaces of the adrenals of normal guinea pigs turned very dark brown, almost black. On the second day of the diet period, there was a noticeable decrease in the intensity of reduction. The intensity of reduction decreased progressively until on the sixth day it was scarcely noticeable. The color of the cut surface changes progressively from a very dark brown, almost black, to a reddish orange on the fifth day. We do not know whether this change in appearance is due to the action of a very small amount of vitamin C or whether it is due to the precipitation of the proteins by silver nitrate. After the sixth day, if any reduction takes place, the extent is so small that it is scarcely evident. After the sixth day, it was noticed that the capsule of the adrenals showed a more marked change in appearance than did the cut surface.

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[1] Szent-Gyorgyi, A., *Biochem. J.*, 1928, **22**, 1387.

[2] King, C. G., and Waugh, W. A., *Science*, 1932, **75**, 357.

[3] Miller, C. O., Siehrs, A. E., and Brazda, F. G., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 636.

[4] Moore, T., and Ray, S. N., *Nature*, Dec. 31, 1932, 997.

[5] Sherman, H. C., LaMer, H. K., and Campbell, H. L., *J. Am. Chem. Soc.*, 1922, **44**, 165.

The animals continued to gain weight until the eleventh day. Autopsies showed no hemorrhagic areas about the joints and sternum, with one exception. Beginning on the eighteenth day, each animal was given 3 cc. of orange juice daily by dropper. On the twenty-second day, one animal was killed and its adrenals examined for reducing power. The sliced adrenal turned black in the silver nitrate solution. The intensity of reduction after orange juice feeding was greater than the reduction in the case of the guinea pigs when fed a normal diet.

A comparative study was made of the intensity of reduction in the adrenals with the age of the animals. Five adult guinea pigs, weighing 380-420 gm., were fed a diet of oats and carrots for 2 weeks. They were killed and their adrenals removed; one from each was sliced and placed in the silver nitrate solution according to the usual method. The cortex, medulla and capsule turned black. Animals weighing 150-190 gm., under similar conditions, give a very dark brown but not a definite black.

Photographs were taken for a permanent record.

It has generally been considered that the guinea pig can store only a small amount of vitamin C, since it develops scurvy. We conclude from our study that the amount of vitamin C stored is even smaller than is generally thought and that the supply is rapidly lost either by excretion or by metabolism. The supply is lost more rapidly than is indicated by the development of gross symptoms of scurvy since the decrease is noticeable on the third day on a scorbutic diet and since by the sixth day it has either fallen to a low constant level or is entirely absent. Since considerable time elapses between the disappearance of vitamin C from the adrenal and the development of the gross symptoms, it seems to us that the gross symptoms of scurvy are secondary changes arising as a result of the altered metabolism of the vitamin C deficient animal. The reduction test is a convenient qualitative method for testing for scurvy and for anti-scorbutic substances. We have found that vitamin C is rapidly returned to the adrenals when orange juice is fed to animals with scurvy. This indicates that the cortex of the adrenals has not changed in scurvy enough to interfere with storage. The gross symptoms disappear as a result of the restoration of normal metabolism incident to vitamin C replacement.

Quantitative studies are in progress for determining the amount of vitamin C in the adrenals under varying conditions.

6636

Spontaneous Duodenal Ulcers in Dogs with Chronic Mild Icterus and Hepatitis.

A. C. IVY, V. L. SCHRAGER AND J. E. MORGAN.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

We reported a method[1] for the plastic reconstruction of the common bile duct in which a viable tube was made from a flap of the pyloric mucosa. The tube was connected to either the gall bladder or the common duct, the gastric orifice of the tube being constructed to prevent regurgitation.

Seven dogs were kept to observe chronic effects. Of the 7, five (Dogs 1, 5, 7, 8, 9) have developed duodenal or gastric ulcers after 3 months. After 3 months all dogs showed an elevated icteric index, even though bile could be detected easily in the feces or gastric contents. On autopsy all dogs except one, which is still alive at 21 months, had varying degrees of biliary cirrhosis and hepatitis. Four of the 7 (Dogs 3, 4, 5, 9) developed ascites.

Very brief protocols of the 7 dogs will be given. Dog 1 died at 9 months from a perforated gastric ulcer and 2 duodenal ulcers. Dog 7 died at 5½ months with a duodenal ulcer. Dog 8 died at 3 months from a perforated duodenal ulcer. Dog 4 developed ascites (5 liters) and died without an ulcer at 7 months. At this time the 3 remaining dogs were started on "gastric mucin" (30 gm. daily by mouth). Dog 3 at 7 months post-operatively had ascites but no ulcer when started on mucin. The ascites disappeared and the icteric index decreased from 5 to 3. This dog is now fat and well at 21 months and is still on gastric mucin. Dog 5 at 7 months had such a huge ascites that he could not stand. He had an ulcer as demonstrated by tarry stools. The ascites disappeared in one month after institution of mucin, the blood disappeared from the stools and the dog became fat. Mucin was stopped. The ascites and icterus returned and the stools became tarry. Mucin was started again and the blood disappeared from the stools and the icterus and ascites decreased. After 3 weeks the dog was etherized and only 300 cc. of ascitic fluid was found. Two duodenal ulcers were present with healing edges, only one of which bled on manipulation. Multiple gall stones of the pigment variety were present in the gall bladder, one of the stones measuring 10x8x7 mm. Dog 9 at 6 months had a slight ascites and an icteric index of six. The stools were not tarry, but ulcer was suspected but not proven by operation. On mucin, the ascites disappeared and the icteric index decreased to 1.5. After 17 months while on mucin, the dog developed cord symptoms, which rendered walking impossible. An enteritis developed and the dog was sacrificed at 18 months. A healed duodenal ulcer was found. The stomach was filled with bile. The cord and brain were examined by a neuro-pathologist, Dr. Weil, who found a chronic spinal meningitis with degenerative changes in the lumbar region. Acute cholecystitis was not present in any of our dogs.

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[1] Schrager, Ivy and Morgan, *Surg. Gynec. and Obstet.*, 1932, **54**, 613.

Spontaneous duodenal ulcer associated with hepatitis has also been observed by Halperin[14] in dogs following plastic surgery on the common duct. Ulcers resulted in 7 of 9 dogs in which incomplete stenosis of the common duct was found at autopsy. Our observations parallel his findings and indicate that biliary stasis is the primary predisposing cause of the ulcer formation.

The association of spontaneously occurring duodenal and gastric ulcer with hepatitis and liver insufficiency in the dog is becoming more and more apparent. Ulcer develops spontaneously in dogs with biliary fistula,[2, 3] common bile duct obstruction,[4, 5] cincophen,[6] borneol,[7] and salicylic acid poisoning,[8] pancreatic duct ligation,[5, 9] partial hepatectomy,[10] Eck fistula,[5, 10] and adrenal insufficiency,[11, 12] in which there is a hepatitis or disturbance of liver function. (In dogs operated according to the Mann-Williamson method, Fauley and Ivy have not found evidence of histologic change in the liver, but liver function tests have not been used.) Whether an hepatic disorder is associated with the occurrence of ulcer in man cannot be stated, since as far as we have been able to find, no studies of liver function and histology have been made in ulcer patients. In this connection it is of interest that Shapiro and Lifvendahl[13] found at autopsy 4 duodenal ulcers in 15 cases of tumors of the extrahepatic ducts, in which there occurs, of course, a progressive icterus with liver damage.

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[14] Halperin, *Surg. Gynec. and Obstet.*, in press.

[2] Berg and Jobling, *Arch. Surg.*, 1930, **20**, 997.

[3] Kim and Ivy, *J. Am. Med. Assn.*, 1931, **97**, 1511.

[4] Mann and Bollman, *Arch. Surg.*, 1932, **24**, 126.

6637

Time Factors Concerned in the Germicidal Action of Ultraviolet Light.

A. BACHEM AND M. A. DUSHKIN.

From the Department of Physiology, University of Illinois College of Medicine.

The effect of radiant energy has long been known to be the result of a certain intensity working in a given period of time, during which period a definite amount of energy is being transferred to the object irradiated. Bunsen and Roscoe[1] have correlated these factors and state that identical effects are obtained if the product of the intensity and time is kept constant. This, however, is merely a modification of the Schwartzschild law which states that intensity multiplied by the time to the exponent K must equal a constant. The factor K is a number slightly less than one, whereas the Bunsen-Roscoe law is a special case where the exponent is equal to one, and holds true for a certain limited range of the factors (intensity

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[5] Ivy and Fauley, *Am. J. Surg.*, 1931, **11**, 531.

[6] Van Wagoner and Churchill, *J. Am. Med. Assn.*, 1932, **99**, 1859.

[7] Quigley and Helm. Unpublished. (3 ulcers in 10 dogs.)

[8] Thomson. Unpublished.

[9] Berg and Tucker, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 68; 1932, **30**, 330.

[10] Bollman and Mann, *Arch. Path.*, 1927, **4**, 492.

[11] Oldberg, *Am. J. Physiol.*, 1930, **9**, 275.

[12] Stewart and Rogoff, 1926, **78**, 693.

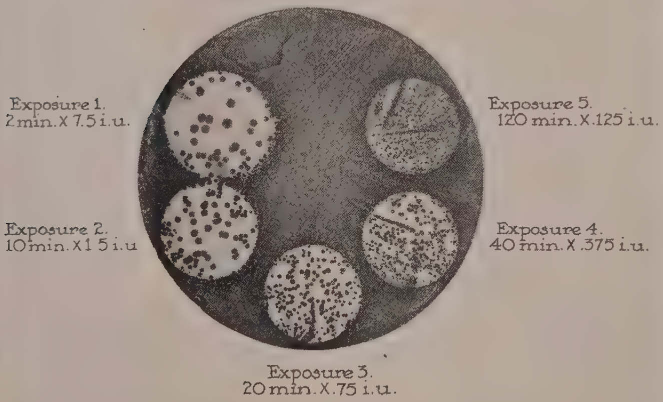
[13] Shapiro and Lifvendahl, *Ann. Surg.*, 1931, **94**, 61.

[1] Bunsen, R., and Roscoe, H., *Ann. Physik.*, 1862, **117**, 529.

and time), whereas the Schwartzschild law must be applied for excessive variations in these factors. Therefore, with marked decrease in the intensity the time must be increased more than the proportional decrease in intensity.

In this experiment we have attempted to ascertain whether the Schwartzschild or Bunsen-Roscoe law is applicable to the destructive effect of ultraviolet light on bacteria. The method consists of spreading in a unicellular layer *B. prodigiosus* on an agar plate. By means of a metal shield, the area of exposure to a water cooled Kromayer lamp can be restricted to a certain field on the plate and at the same time protect the remaining portion. Throughout the experiment the energy applied was constant, but the intensity decreased from 7.5 to 0.12 $\frac{\text{cal}}{\text{cm}^2\ \text{min}} 10^{-4}$, whereas the time was increased in an inverse proportion to the intensity, *i. e.,* from 2 to 120 minutes. The result as indicated in Fig. 1 shows a greater biological effect at short exposures to high intensities and suggests that increased rather than identical energy applications are needed in prolonged exposures to obtain similar biological activity. This would demonstrate the validity of the Schwartzschild law. As a control we performed the same experiment but reversed the order of exposure

Figure 1.
Constant energy application of 15 $\frac{\text{cal.}}{\text{cm}^2} 10^{-4}$ with variable time and intensity factors.



i.u. = intensity unit = $\frac{\text{cal.}}{\text{cm}^2\text{min}} 10^{-4}$

and started with low intensities over prolonged periods. This gave us a diametrically opposed result with greatest biological activity during long exposures with low intensities, a finding which is neither in holding with the Schwartzschild nor Bunsen-Roscoe law. (Fig. 2.)

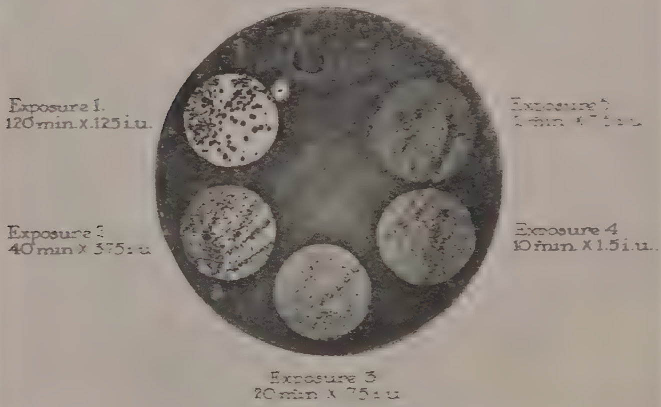


Figure 2.
Constant energy application of 15 $\frac{\text{cal.}}{\text{cm}^2}$ 10^{-4} with variable time and intensity factors.

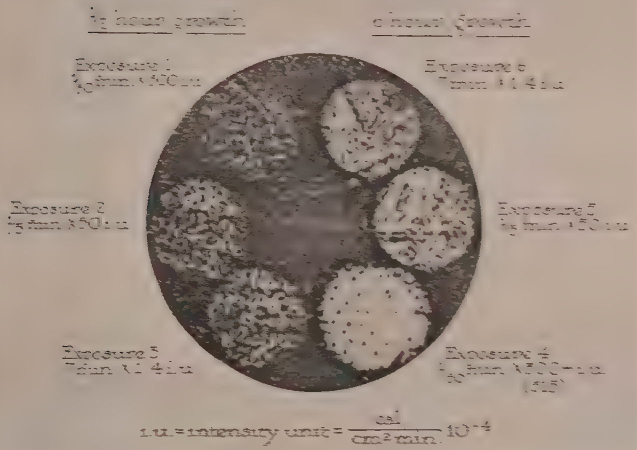
i.u. = intensity unit = $\frac{\text{cal.}}{\text{cm}^2\ \text{min.}}$ 10^{-4}

Therefore, we concluded that the intensity and duration of the exposures were not the only conditions involved, but that some other factor which must be inherent in the bacterial culture itself is also to be considered. To prove this we systematically studied the sensitivity of bacteria to equal amounts of ultraviolet light (keeping time and intensity constant) and found that there was a larger or smaller sensitivity depending upon the duration between the time of plating and time of exposure, the bacteria being kept at room temperature, 70° F.

Time interval	% killed
0	40
2 hr.	94
4 "	97
6 "	98
8½ "	96
10 "	51

This difference in sensitivity at different periods of bacterial

Figure 3.
Constant energy application of $10 \frac{\text{cal.}}{\text{cm}^2\text{min.}} 10^{-4}$ with variable time and intensity factors.



growth is more fully shown in Fig. 3, in which exposures 1 and 4, 2 and 5, 3 and 6 are identical, but 1, 2, 3 were made after 1½ hr. growth and 4, 5, 6 after 6 hrs. of growth. In our next experiments we selected periods of maximum sensitivity, during which no perceptible change of resistance occurs. By means of a sector we used a ratio of 1:100 and obtained identical results, which aid in ascertaining the non-validity of the Schwartzschild law but sustains the Bunsen-Roscoe law.

These experiments were of importance to us in that our study of the spectral distribution of the germicidal property of ultraviolet light forced us to compare short and long exposures of varying intensities. Thus our time comparisons had to be based on the validity of either the Bunsen-Roscoe or the Schwartzschild law. Liechti[2] reported that the Schwartzschild law is applicable to bacterial destruction by ultraviolet light, while Gates[3] agrees with Coblentz and Fulton[4] in maintaining the non-applicability of the Bunsen-Roscoe law but claims the validity of the Schwartzschild law. We, however, maintain that the Bunsen-Roscoe law is applicable, but only for those periods in which there is no change in the bacterial sensitivity. The results of the authors quoted are probably incorrect in that they have committed our initial error of studying the Schwartzschild factor during a period of decreasing sensitivity; an error, however, which we corrected by means of our controls.

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[2] Liechti, Adolf, *Strahlentherapie*, 1929, **33**, 1.

[3] Gates, Frederick L., *J. Gen. Physiol.*, 1929, **13**, 249.

[4] Coblentz, W. W., and Fulton, H. R., *Sci. Papers Bur. Stand.*, 1924, **19**, 495.

6638

Observations on Administration of Chondroitin in Peptic Ulcers.

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From the Department of Physiology and Pharmacology, Northwestern University Medical School.

The effectiveness of mucin in the treatment of peptic ulcer has usually been ascribed to a local coating effect by which it protects the mucous membrane, its acid combining power, or to an inhibition of peptic digestion. Because we observed beneficial effects from mucin in dogs with liver injury,[1] where the results could hardly be ascribed to a local action, we became interested in its possible systemic effects. Mucin is a glycoprotein, a combination of protein with a carbohydrate radical (the prosthetic group). Chondroitin sulphuric acid, the prosthetic group of chondromucoid, is very similar in composition to the mucoitin that is present in mucin and benefits animals with experimental liver injury as does mucin. Chondroitin and mucin both contain glucuronic acid, which is known to be used by the liver for the detoxification of phenol derivatives and other substances. We felt that a study of a group of patients with peptic ulcer treated with chondroitin might indicate whether any of the action of mucin could be ascribed to a systemic effect produced by the glucuronic acid that it contains. Determinations of the uronic acid content of various batches of commercial mucin by Link's modification of the Lefevre and Tollens method[2] gave results varying from 1.2 to 2.5% glucuronic acid. The theoretical glucuronic acid content of chondroitin is 37.6%; the chondroitin that we have used (at first prepared by us, later made for us by the courtesy of Chappel Bros. Inc.) varied from 95 to 98% pure. We have used 3 to 6 gm. of chondroitin per patient per day in divided doses; this contains an amount of glucuronic acid equivalent to that in the usual daily dose of mucin. Chondroitin is not a gummy material, could hardly be expected to coat the mucous membrane, and was given in the form of the free acid; its action, therefore, cannot be attributed to any local effect.

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[1] Crandall, L. A., Roberts, G. M., and Gibbs, J. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1082.

[2] Lefevre and Tollens, *J. Am. Chem. Soc.*, 1930, **52**, 775.

Twenty-two patients with peptic ulcer have been observed over periods of 2 to 9 months. In 20 cases the ulcer was in the duodenum; one case had an ulcer of a gastro-enterostomy stoma, and one had both lesser curvature and duodenal ulcers. In all but 2 cases the ulcer was verified by X-ray, both of these cases presented the classic ulcer syndrome. The history of ulcer prior to chondroitin therapy varied from 1 to 18 years. All the patients had previously been on alkali treatment, which completely relieved the distress in 4 and gave partial relief in all but 4 during the most recent period of alkalinization.

Treatment was ambulatory in every case, and was carried out in the Dispensary of the Northwestern University Medical School and in the ulcer clinic of Dr. Singer at Cook County Hospital. We wish to express our appreciation to Drs. Snorf and Mahle of Northwestern and to Dr. Singer for their most helpful cooperation. In almost every case the patient was either already on a bland diet or was much restricted in choice of food by financial conditions. The plan we endeavored to follow was to make no change other than the administration of chondroitin, except that when the patient was taking alkaline powders these were gradually eliminated during the first 3 or 4 days of treatment. In most cases the patient was already on a modified diet or had voluntarily resumed a bland diet with reappearance of distress, so that only occasionally was any appreciable modification necessary. Such ambulatory treatment allowed less supervision of the patient than would have been possible had hospitalization been employed, but it was felt that maintaining all other factors as constant as possible permitted more accurate inferences as to the effectiveness of chondroitin.

Of the 22 patients, 10 were relieved. Relief is defined as a practically complete absence of ulcer symptoms during the period of treatment except for recrudescence of distress following major dietary indiscretions. Of the remaining 12 cases, 6 appeared to show some benefit. Six of the 10 cases that were relieved were subjected to withdrawal of chondroitin for a short period during the first two months of therapy and in all of them the typical ulcer distress returned, to disappear again when treatment was resumed. Four of the 10 cases that responded had not been completely relieved by alkali therapy in the few months preceding chondroitin, although all 10 had shown remissions on alkali at some time during their ulcer history. The average time for disappearance of symptoms in 10 patients was 7 days. In those cases that were rechecked by X-ray, a decrease in spasm was noted. It is worthy of note that 5 of the patients complained of headache, which was usually more frequent and severe during those periods when ulcer distress was present. This headache was not related to previous alkali therapy. In these 5 cases the headaches promptly disappeared when chondroitin was administered, in fact the ulcer distress usually persisted longer than the headache.

It is our impression that the administration of chondroitin produced definite improvement in about 45% of our series of patients, who were in general under unfavorable conditions of diet. The fact that distress disappeared on therapy, reappeared when treatment was discontinued, and disappeared again when chondroitin was resumed is significant. Most of the patients also exhibited a gain in weight and improvement in general condition.

These results are obviously much inferior to those reported following the administration of neutral gastric mucin containing equivalent amounts of glucuronic acid. It appears probable that the systemic action of the prosthetic group of the mucin molecule (mucoitin) accounts for a part of the effectiveness of mucin in the treatment of gastric ulcers, perhaps by supplying building stones for the formation of mucus in the body of the patient. It is highly possible that the administration of large amounts of glucuronic acid, a substance of considerable biologic importance, may be beneficial in a manner not yet clearly understood. There is no evidence that the body finds any difficulty in producing galactosamine or glucosamine in amounts adequate to its needs; if this is correct glucuronic acid is the portion of the chondroitin molecule which might be expected to be physiologically active.

Our results also indicate that the local action of mucin is a factor in promoting the healing of peptic ulcer, but a definite statement on this point is impossible until the rôle of the various amino sugars can be determined.

6639

Intraintestinal Pressure and Absorption from the Intestine.*

G. J. DOBYNS AND C. A. DRAGSTEDT.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

The well recognized changes in the blood chemistry in intestinal obstruction coupled with the clear demonstration of the fatal effects of the loss of gastric or pancreatic juice have brought about a modification of the older theories which held that the cause of death in intestinal obstruction was predominantly if not exclusively a toxemia. A definitely tangible and measurable syndrome of dehydration, hypochloremia, etc., can be shown to indicate, if not determine, the fatal issue in many cases. The conception that a toxemia arises from the absorption of toxic substances from the intestinal lumen has been largely restricted to cases of strangulation obstruction, with considerable skepticism that it plays any rôle even here. However, a number of observations in the literature seem to be reasonably interpreted on the basis that toxic absorption can and does occur, among them the reports of Stone and Firor,[1] Läwen,[2] Dragstedt,[3] Gatch, Owen and Trusler,[4] Roberts and Crandall,[5] and Tonnis and Brusis.[6] In most of these reports such toxic absorption seemed to be conditioned in part on an abnormal intraintestinal pressure either developing as a result of the obstruction or artificially produced.

We have attempted to study the influence of such degrees of intraintestinal pressure as may occur in intestinal obstruction upon the absorption of certain toxic and non-toxic substances from the intestinal lumen. All of the experiments were performed upon dogs anesthetized with sodium barbital.

In a series of 5 dogs efforts were made to note any variability of lymphatic absorption by a study of the thoracic duct lymph, after dyes, colloidal silver, emulsified fats, etc., were introduced into a

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* Aided by Grant No. 200 (Van Zwalenburg Fund) American Medical Association.

[1] Stone, H. B., and Firor, W. M., *Trans. South. Surg. Assoc.*, 1924, **37**, 173.

[2] Läwen, A., *Z. fur Chirurg.*, 1927, **54**, 1037.

[3] Dragstedt, L. R., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 239.

[4] Gatch, W. D., Owen, J. E., and Trusler, H. M., *West. J. Surg. Obstet. and Gynec.*, 1932, **40**, 161.

[5] Roberts, G. M., and Crandall, L. A., *Arch. Int. Med.*, 1932, **50**, 150.

[6] Tonnis, W., and Brusis, A., *Deut. Z. f. Chirurg.*, 1931, **233**, 133.

segment of bowel which was then subjected to varying levels of distention either with air or water pressure. No definite effects were observed. Inasmuch as short segments of bowel (20 to 40 cm.) were used, such negative results do not preclude the possibility that variations might appear if the whole or major portion of the intestine were employed.

In the second series of dogs, active pharmaco-dynamic agents that are not normally readily absorbed from the intestine were introduced into intestinal loops and these then distended to various pressure levels. Histamine, epinephrine and acetylcholine were used. Histamine in doses up to 100 mg. had little or no effect whether the intestinal loop was distended or not. After prior irrigation of the loop with 50% alcohol, absorption of histamine was indicated by the subsequent fall in blood pressure, and this was increased by mild degrees of distention (20 to 40 cm. water pressure). Distention had apparently no effect on epinephrine absorption. Likewise in only one of 5 experiments with acetylcholine was there any definite evidence that distention of the intestinal loop augmented its absorption.

Gatch and his coworkers[4] call attention to a certain amount of transperitoneal absorption that may occur from intestinal loops distended by rather high pressures, pressures sufficient to arrest most of the mesenteric blood flow. These observations were confirmed by us in the following experiments in a third series of dogs. Two dogs, anesthetized with sodium barbital, were held closely together on a dog board so that an isolated loop of intestine from one dog could be introduced into the peritoneal cavity of the second dog without interfering with the mesenteric blood supply to the loop. Active pharmaco-dynamic agents which are normally readily absorbed (nicotine, synephrin, pilocarpine, ephedrine, and sodium nitrite) were introduced into the intestinal loop which was then distended to various pressure levels. Such mesenteric absorption as occurred would be manifested by the effects produced in the dog to whom the intestinal loop belonged, while such transperitoneal absorption as occurred would be manifested in the reactions of the second dog. It was observed that with all pressure levels below that of the diastolic blood pressure (of the dog to whom the intestinal loop belonged) evidences of mesenteric absorption alone resulted. Variations in pressure did not appear to alter appreciably the rate of this absorption. When the pressure was kept above that of the diastolic blood pressure, no effects whatever were noted in the dog to whom the loop belonged, *i. e.,* mesenteric absorption was completely arrested. Evidences of transperitoneal absorption occurred in 2 of the 5 experiments in which nicotine was employed, in one of the 5 experiments in which pilocarpine was employed, but in no instance with the other drugs employed. In the 2 positive experiments with nicotine, the dog to whom the loop belonged had died 10 and 20 minutes respectively before evidences of transperitoneal absorption of nicotine were apparent. In the case of the positive experiment with pilocarpine, the distention had been maintained for approximately one hour. These positive results were therefore apparently conditioned by devitalization of the loop.

These observations seem to warrant the following conclusions: Variations in intraintestinal pressure have little effect upon the mesenteric absorption of readily absorbable substances until the pressure reaches that of the diastolic blood pressure, when such mesenteric absorption ceases. Moderate distention occasionally facilitates the absorption of substances not normally readily absorbed. Transperitoneal absorption may occur but is very slow and probably insignificant from viable intestine, while it may become significant from a devitalized segment of bowel subjected to distention.

6640

Passage of Exogenous Bacteria Through the Wall of an Isolated Stomach.

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From the Department of Bacteriology and Preventive Medicine, University of Illinois, College of Medicine, and Research Laboratories of the State Department of Public Health.

Nedzel and Arnold[1] showed the passage of exogenous bacteria from the small intestines into the blood circulation. Boone and others[2] proved the passage of the same bacteria into the portal circulation from the small intestines. Fisher[3] showed the same for yeast. Shuger and Arnold[4] gave evidence of absorption of exogenous bacteria from the large intestine. Fisher[5] does the same for yeast. Nedzel and Arnold[6] prove the passage of bacteria through the wall of the gallbladder. Tsuji[7] states that bacilli introduced into the gallbladder can invade the blood, bile and urine through the mucous membrane of the gallbladder and that the invasion is more powerful in cases of pathological changes of the gallbladder wall.

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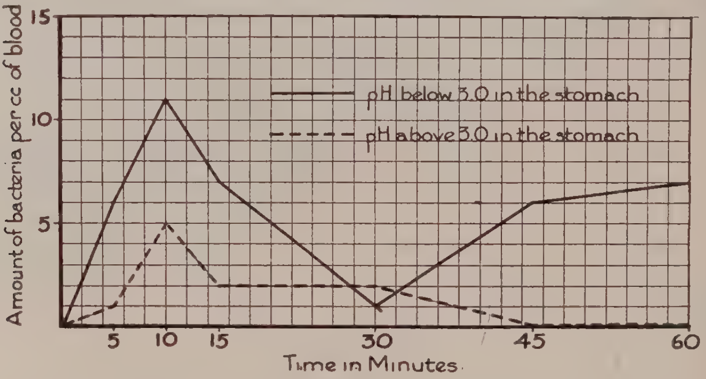
[1] Nedzel, A. J., and Arnold, L., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 358.

[2] Boone, T., Chase, E., Brink, E., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 113.

The permeability of the stomach was studied in the following manner: 20 dogs, fasted for 24 hours, were used; all were given nembutal anesthesia. The abdomen of each dog was opened, the duodenum exposed and tied off near the pylorus, carefully avoiding the possible ligation of the large blood vessels in this region. An opening was made in the duodenum adjacent to the ligature. A row of ligatures was placed around this opening to prevent bleeding. We were able to introduce material into the stomach and also to obtain specimens with a pipette.

The experiments fall into 2 groups. One group (11 dogs), where we introduced into the stomach of each dog a suspension of *B. prodigiosus* (washings of one agar plate of 24 hours growth) in 50 cc. of an isotonic saline solution where the pH of the stomach contents after introduction of this suspension was below 3.0. In the other group (9 dogs) the same amount of *B. prodigiosus* was used but they were suspended in 50 cc. of an alkaline buffer solution. The further technic was the same for both groups, namely: Four drops of blood were drawn by means of a sterile syringe from a right gastro-epiploical vein at 5, 10, 15, 30, 45, and 60 minute time intervals, and plated on agar. Simultaneously a specimen of stomach



GRAPH 1.

Ordinate—amount of bacteria per cc. of blood. Abscissa—time in minutes.

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[3] Fisher, V., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 948.

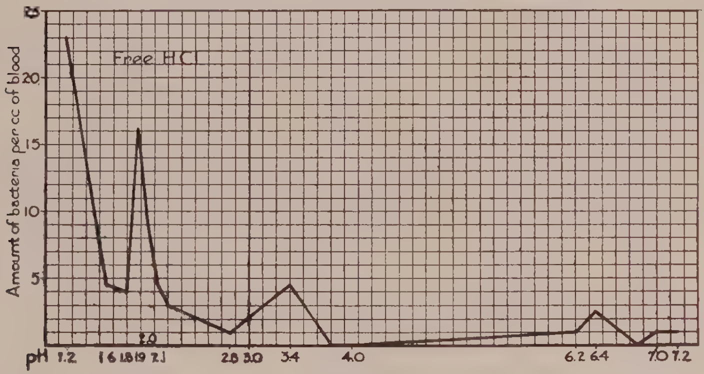
[4] Shuger, M., and Arnold, L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 494.

[5] Fisher, V., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 490.

[6] Nedzel, A. J., and Arnold, L., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 366.

[7] Tsuji, Joichi, *Biological Abstract,* 1932.

contents was removed with a sterile pipette and the pH determined (La Motte hydrogen ion testing set). The plates were recorded after 48 hours' incubation.



GRAPH 2.

Ordinate—amount of bacteria per cc. of blood.
Abscissa—hydrogen ion concentration.

The results of experiments are presented on the 2 accompanying graphs. The first graph shows the amount of bacteria per cubic centimeter of blood that passed through the wall of the stomach into the blood of the right gastro-epiploical vein at a certain time interval. The second graph presents the results of the same experiments, but here we have disregarded the time factor. On this graph we see how the absorption of bacteria goes all the way through in waves and that this absorption is much greater in a stomach with the contents of free hydrochloric acid. Lower pH, higher absorption of the bacteria and vice versa.

Conclusions. 1. The exogenous bacteria are absorbed from an isolated stomach into the blood. 2. The greatest number are obsorbed in the first 15 minutes after the introduction of bacteria into the stomach. 3. The lower pH of the stomach contents, the higher absorption of the bacteria.

6641

An Intracutaneous Methylene Blue Reaction.

W. F. PETERSEN AND B. APPELMAN.

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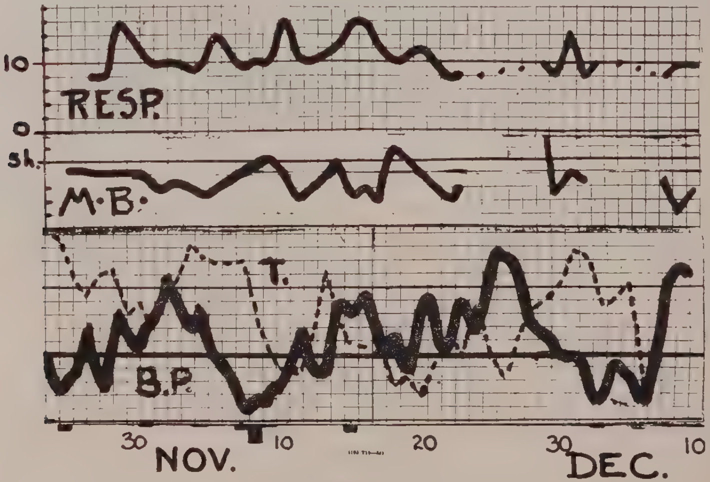
We have made use of intracutaneous injections of methylene blue to determine relative oxidation-reduction potential of the epithelium, using 2 different dilutions (1-1000 and 1-4000) in distilled water and injecting approximately 0.01 cc. by means of a tuberculin syringe. Two different dilutions are used merely as a matter of convenience for different patients in whom the oxidation reduction potential may show great fluctuations.

When so injected the disappearance time should afford some index of the rapidity of reduction and we have carried out a large number of observations on normals as well as on patients with various diseases.

In a study of individual patients as well as of normal individuals observed day by day, an appreciable difference in the disappearance time can be determined. In an effort to determine the cause of these differences we have noted that meteorological factors apparently are closely associated. Thus the barometric depression of the cyclonic wave is followed by an increase in reduction time (greater oxida-



GRAPH 1.

tion) while a rise in barometric pressure is associated with a reduction in the disappearance time. The respiratory rate of the person (even when kept under uniform temperature conditions) is frequently opposite in trend, *i. e.*, the rate increases when the methylene blue disappearance time is diminishing.

We append a graph of an autonomically unstable patient on whom observations were made from a period of October 25th to December 10th. In this graph the upper curve represents the respiratory rate taken in the morning. It will be noted that it ranges from 8 to 16 per minute. The curve immediately below labeled M.B. represents the disappearance time of the injected dye. The two curves at the bottom of the chart are of meteorological observations. The heavier line marked B.P. being the Chicago barographic record and T, the dotted line, the average daily temperature.

The disappearance time of the injected methylene blue forms almost a mirror image of the barograph.

Without entering into a discussion of the underlying mechanisms we wish at this time merely to call attention to the probable usefulness of methylene blue as an indicator of oxygen reduction potential when so injected.

6642

Measurement of the Action-Potentials in the Peripheral Nerves of Man Without Anesthetic.

EDMUND JACOBSON. (Introduced by A. J. Carlson.)

From the Physiological Laboratory, University of Chicago.

Our method to secure records of nerve currents in man is to insert one or both electrodes directly into a peripheral nerve. Two types of electrodes are used: (1) A fine wire of platinum-iridium is inserted into the ulnar nerve while a second wire is inserted under the skin at the point of the elbow. The second wire is the so-called indifferent electrode. (2) The tip of a hypodermic needle containing 2 very fine and well-insulated wires, the electrodes, is inserted into the ulnar nerve. The needle itself is connected to ground, while the electrodes are connected with the amplifier input. Possible advantages of this type of electrode over the well-known form of Adrian are under investigation. Electrodes described under (2) are particularly suitable for measuring action-potentials from a limited area of nerve tissue immediately surrounding the electrodes.

Measurements are made with apparatus previously described,[1] including an amplifying equipment and string galvanometer so that if one microvolt at 60 cycles is impressed upon the input terminals of the amplifier a fairly steady deflection of the string of about 2.4 cm. is obtained. At this setting, if a short-circuiting line is placed across the input terminals, the string shows irregular excursions generally not exceeding 0.6 or 0.7 cm.

Whether the electrode or electrodes actually are in the nerve can be determined as follows: (1) As the needle penetrates the nerve, the subject reports a sudden pang in the ulnar portion of the palm and in the ulnar 2 fingers, sometimes including the middle finger. (2) Leads from the electrodes are disconnected from the amplifier and connected into a circuit containing a dry cell and a rheostat, so that a slight stimulating current can be sent through the tissues between the points of the electrodes. That the electrode or electrodes lie in the nerve tissue is shown by the stimulation causing sudden abduction of the hand ulnarwards. If the wire or needle presumed to be in the nerve is withdrawn so that it penetrates the skin and underlying tissues only several millimeters deep, or if it is pushed in completely but not in the same direction as originally (*e. g.*, hypodermically) stimulation with the slight current fails to cause motor effects characteristic of ulnar motor fiber action; considerable increase of such current likewise fails. (3) With the electrodes in the nerve and connected in the amplifying circuit, the subject upon signal makes slight movements with muscles whose motor supply is solely from the ulnar nerve; for instance, he flexes ever so slightly the terminal phalanges of the ulnar 2 fingers. Thereupon the string movements show the presence of marked action-potentials. A control test is made upon flexing the terminal phalanx of the thumb, when no such action-potentials appear.

The subject, with electrodes suitably attached, lies on a couch with eyes closed, and is requested to relax fully. During a test period, action-potentials are recorded with the photographic paper moving at the rate of one inch per second or at a higher speed. To avoid securing too long a photograph in experiments which last half an hour or more, the camera motor is turned on and off at 3 regular intervals per minute by a clockwork operating a relay switch. Control records are taken upon short-circuiting the input leads of the amplifier, thus eliminating the subject from the circuit. Calibrating records are made, for example, by impressing one microvolt at 60 cycles directly upon the input terminals of the amplifier and, again, in series with the subject, so that the impressed microvoltage passes through the electrodes and the tissues between them.

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[1] Jacobson, E., *Am. J. Physiol.*, 1930, **91**, 567; 1931, **97**, 200.

Measurements can thus be made from photographs of the nerve action-potentials of an individual, normal or diseased. The results can be stated as the total microvoltage for the entire period, as the average microvoltage per second, or as the average peak microvoltage per second. The changes in microvoltage in the electrodes during the period can be represented in a graph. Possible clinical applications will be apparent.

Following the period of quiet, various stimuli are applied such as might excite the subject; or he engages in some exciting activity while action-potentials are measured. Stimuli include sudden noises, disagreeable sounds, the application of heat and exposure of bright light upon the closed eyelids.

Subjects who have had prolonged training to relax, so far as tested up to date, generally show a line of potential near zero when relaxed. These findings accord with those secured with a larger group of trained individuals in whom measurements have been made with the electrodes inserted in muscle. As an illustration, when one such subject spoke or counted out loud, no marked deviation from zero was noted in action-potentials recorded from the ulnar nerve. But when requested to relate about his father's recent death, a great increase in action-potentials occurred. Upon ceasing his account, following the instruction to relax, the action-potentials decrease in gradual linear manner. After almost zero potential has been regained for several minutes, the same requests are followed by repetition of similar phenomena.

Pacific Coast Section.

Stanford University Medical School, February 8, 1933.

6643

Summation of Contractions in the Turtle Heart.

HENRY M. LEE. (Introduced by H. F. Blum.)

From the Division of Physiology, University of California Medical School, Berkeley.

The phenomenon of summated contractions cannot be obtained in cardiac muscle under ordinary conditions. Mines,[1] however, found such an effect in the hearts of *Torpedo ocellata* which had been removed from the body for some time. He related this exception to the fact that the extraordinarily long refractory period of the heart had been shortened under the experimental conditions. Thus a second stimulus occurring sufficiently soon after a first would allow summation of one contraction upon another. Burridge[2] found that calcium salts greatly favored summation in frog ventricle muscle. Alcohol, ether, and alkali also had a somewhat similar effect.

In this laboratory while studying the vagus effects on the turtle heart it was discovered that in a limited number of cases extracontractions could be summated upon normal contractions during vagus inhibition.

In these investigations hearts of *Chrysemys elegans* and *Chrysemys troosti* were used. It was found that summation of contractions was possible in either auricle with homolateral vagus stimulation, but the right auricle because of its greater size and contractility always demonstrated the phenomenon to an advantage. Accordingly, the right auricle was used in most of the experiments, its contractions being recorded on a smoked surface by means of a sensitive isometric lever. Since the negative chronotropic effect of the right vagus in this genus generally overshadows its negative inotropic action, it was found necessary to maintain rhythm of the auricles artificially during inhibition. This was accomplished by sending rhythmical break shocks into the non-recording auricle, thus maintaining contractions in all parts.

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[1] Mines, G. R., *J. Physiol.*, 1913, **46**, 1.

[2] Burridge, W., *J. Physiol.*, 1913, **47**, 31.

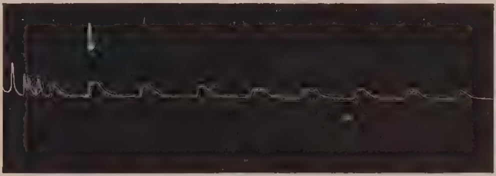

FIG. 1.

Representing summation of contractions in turtle auricular muscle. The left vagus and left auricle were used in this illustration.

Fig. 1 is a characteristic result showing that an extra-systole during the period of vagus stimulation will produce a total height of contraction surpassing that of the regular driven beat. This is true only when this extra-contraction occurs within a short time after the normal, approximately 1.5 seconds in most cases at room temperature.

In order to obviate the difficulties of recording such slight amounts of tension on the smoked surface an optical lever was employed and records of the amplitude of contractions were read directly from the excursions of a beam of light upon a centimeter scale. The effects were the same as those illustrated graphically and are shown in Table I. As the interval between the normal and extra-contractions approaches 1.5 seconds, the amplitude of the contractions of the normal and extra beats becomes approximately equal.

TABLE I.

Height of regular beat, 0.7 cm.

Total height of extra-contraction	Interval between regular and extra-contraction
cm.	sec.
1.1	0.2
0.9	0.4
0.7	1.4

It will therefore be apparent that summation of contractions is possible in turtle atrial muscle during vagus inhibition. The observed shortening of the refractory phase may be partially responsible for the described phenomena. Perhaps a sufficient increase in the rate of the recovery process together with a decrease in the refractory period may be the underlying cause. Further work along this line remains to be done.

6644

Physiological Effects of Light

HAROLD F. BLUM.

From the Division of Physiology, University of California Medical School, Berkeley.

The destructive effects produced in living systems by ultraviolet light are in many cases quite similar to effects produced by visible light in living systems sensitized by dyes (photodynamic action). As examples may be cited: the hemolysis of red blood cells; the production of erythema, pigmentation, and destructive lesions of the skin of mammals; muscle responses, etc. These similarities suggest a common causal mechanism.

The writer[1] has called attention to the fact that the photodynamic and ultraviolet effects can be differentiated, in so far as they have been studied, by the fact that the former take place only in the presence of oxygen, whereas the latter take place quite readily in the absence of oxygen. Nevertheless the thermodynamic considerations given below indicate the possibility of a common mechanism.

In investigations of the photochemical reactions of fluorescein dyes in this laboratory it has been found that hydrogen peroxide is formed when aqueous solutions of these dyes are irradiated with visible light in the presence of molecular oxygen, and as has been shown by the writer,[2] it is most probable that the production of peroxide is a step in the destruction of living systems sensitized by these dyes.

The principal reaction involved would appear to be:

(1) $2\,H_2O\,(1.) + O_2 = 2\,H_2O_2\,(aq.);\ \Delta F_{298} = 50180$ cals.; $\Delta H = 46000$ cals.[3]

Obviously this reaction cannot proceed very far in the direction indicated, and at equilibrium the concentration of H_2O_2 present would be approximately 10^{-18} M. Since in the above mentioned experiments H_2O_2 was produced in concentrations up to 10^{-3} M, it is obvious that we are not dealing with a thermal reaction, the rate of which is increased by the action of light, but one in which the energy is supplied directly by the light.

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[1] Blum, H. F., *Physiol. Rev.*, 1932, **12**, 23.

[2] Blum, H. F., *Biol. Bull.*, 1930, **58**, 224; **59**, 81.

[3] The data for the calculation of ΔF and ΔH in this paper were obtained from *Thermodynamics*, Lewis and Randal (1923), McGraw Hill, New York; and *International Critical Tables*. The values of ΔH are taken to the nearest 1000 cal., which is sufficient for our purposes.

We may represent the reaction as follows:

$$(2)\quad D + h\nu = D'$$

Where D is the dye molecule, D′ an activated molecule, and $h\nu$ the light quantum.

$$(3)\quad D' + H_2O = D + H_2O'; \text{ or } D' + O_2 = O_2' + D$$

Indicating the transference of the energy of activation from D′ to O_2 or H_2O.

$$(4)\quad 2\,H_2O + O_2' = 2\,H_2O_2$$

It is possible that an intermediate peroxide of the dye may be formed, but if so, our experiments would indicate that it must proceed very rapidly to H_2O_2 and dye.

$\Delta H/N$ ($N =$ Avagadro's number) is in this case the energy which must be supplied per molecule by light or the minimum value for the quantum which is capable of bringing about this type of reaction.

Under such conditions:

$$\frac{\Delta H}{N} = h\nu$$

when h is Planck's constant and ν the frequency of the radiation, and since

$$\nu = \frac{c}{\lambda}$$

where λ is the wave length and c the velocity of light

$$\lambda_{max} = \frac{c\,h\,N}{\Delta H}$$

λ_{max} representing the maximum wave length which would supply sufficient energy for the reaction.

$\Delta H = 46000$ cals. for reaction (1) and, substituting the proper numerical values:

$$\lambda_{max} = \frac{3 \times 10^{10} \times 6.56 \times 10^{-27} \times 6.06 \times 10^{23}}{46000 \times 4.185 \times 10^{7}} = 6.195 \times 10^{-5} \text{ cm.} = 6195\text{Å}$$

Thus it will be seen that light of wave lengths less than 6200Å is capable of bringing about reaction (1), but not light of longer wave lengths. Most of the visible spectrum and all the absorption spectra of the fluorescein dyes are included below this value so that reaction (1) is possible as the chemical mechanism for the production of the photodynamic effects.

The reaction:

$$(5)\quad 2\,H^+ + O_2 = H_2O_2\,(aq.);\ \Delta F = -31470 \text{ cals.}$$

has a large negative free energy but could not account for the concentration of H_2O_2 formed in our experiments, since the concentration of H^+ limits the concentration of H_2O_2 which can be formed to 10^{-14} M.

The mechanism:

$$(6)\ D' + O_2 = 2\,O + D;\ \Delta H = 150{,}000 \text{ cals.}$$
$$(7)\ H_2O + O = H_2O_2$$

is ruled out since reaction (6) would require 150,000 cals., which represents a maximum wave length far below the visible region.

Assuming the possibility that ultraviolet effects, like the photodynamic effects, are due to the production of H_2O_2 the following reactions are possible:

$$(8)\ 2\,H_2O\ (1.) = H_2O_2\ (aq.) + H_2;\ \Delta F_{298} = 81650 \text{ cals.};\ \Delta H = 91000 \text{ cals.}$$
$$\text{or}\ (9)\ 2\,H_2O\ (1.) = H_2O_2\ (aq.) + 2\,H^+$$

These reactions do not involve molecular oxygen, as is required by the experimental evidence. $\Delta H = 91000$ cals. and calculating as above we find 3205 Å as the longest wave length at which this reaction can occur.

It may be considered highly suggestive that the value of 3200 Å very closely approximates the upper limit for the known destructive ultraviolet effects in living systems. Interestingly enough, it also includes below it the region of anti-rachitic effects and that for the activation of ergosterol.

In reaction (8) it might be expected that the H_2 molecule, if formed, would combine with a hydrogen acceptor in the tissues. Since water does not absorb very strongly above 2000 Å, the mechanism would probably necessitate a sensitizer which we must assume as some compound present in the tissue; this compound might also serve as the hydrogen acceptor.

The reactions:

$$3\,OH^- = HO_2^- + H_2O\ (1.);\ \Delta F_{298} = 40195 \text{ cals.};\ \Delta H = 61000 \text{ cals.}$$
$$\text{and}\ 2\,OH^- = H_2O_2\ (aq.);\ \Delta F_{298} = 59300 \text{ cals.};\ \Delta H = 65000 \text{ cals.}$$

could occur up to 4700 Å and 4400 Å respectively. However, the probability of an activated sensitizer molecule colliding with an H_2O molecule as against its collision with an OH^- ion might be expected to be about 55×10^7. In any event the concentration would be limited by the concentration of OH^- ion, so that these reactions could probably not account for H_2O_2 production in effective concentrations.

The above reactions are offered to call attention to an interesting possibility. Whether there is a definite relation between photodynamic and ultraviolet effects and whether H_2O_2 is or is not an immediate causal factor in the latter can only be determined by further experimental study. Since the photodynamic effects are probably the resultants of 2 mechanisms, a photo and a dark reaction (see Blum[2]), certain discrepancies in the photodynamic and ultraviolet effects are to be expected. Numerous other mechanisms for ultraviolet effects are, of course, possible, and the mechanism suggested can only be considered as a working hypothesis. Experiments are in progress in this laboratory to test the point.

6645

Electromigration of Iodobismuthite in Colloidal Systems.

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Electromigration of the complex ion (BiI_5) of sodium iodobismuthite (Na_2BiI_5) in colloidal systems is of interest in connection with the absorption and cerebrospinal penetration of this electronegative bismuth in antisyphilitic medication.[1] The red (BiI_5) ion migrates to the anode in aqueous, alcoholic and glycolic media.[2] The question of cerebral and spinal fluid penetration hinges, partly at least, on another question, whether the unchanged complex ion (BiI_5) can migrate in colloidal systems analogous to those of the body, such as serum and a gel. Direct test of migratibility in living tissue and blood is practically impossible. Therefore, a special electrolytic cell, which also acted as a model, containing serum, agar-gel, and iodobismuthite was used.

The anionic character of the bismuth in iodobismuthite was originally demonstrated with bare platinum electrodes.[2] However, such electrodes require constant attention in order to avoid decomposition of the labile complex (BiI_5) ion. Therefore, the use of KCl-agar bridges in the anode and cathode chambers was suggested, since their use would prevent loss of the electrical charge on, and consequent decomposition of, the complex (BiI_5) ion. This arrangement also made it practically feasible to test the migration, if any, of the (BiI_5) ion in colloidal systems, *i. e.,* into serum and from serum into the agar-gel. The analogy to conditions prevailing in the intramuscular administration of iodobismuthite (iodobismitol) was almost perfect. That is, in the model, the serum phase corresponded to the lymph and plasma (blood) with which iodobismuthite comes in contact when injected intramuscularly, and the agar-gel phase, to the tissues and brain to be penetrated. Such an electrical field does not exist, of course, in the living fluids and tissues. The chief importance of this test is the demonstration of the possible migration and survival of the complex (BiI_5) ion in aqueous colloidal systems without hydrolysis, or at least with retarded hydrolysis.

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[1] Hanzlik, Mehrtens, Gurchot and Johnson, *J. Am. Med. Assn.*, 1932, **98**, 537; Hanzlik and Spaulding, *Am. J. Syph.*, 1932, **16**, 335; Hanzlik, Mehrtens and Spaulding, *Am. J. Syph.*, 1932, **16**, 350.

[2] Hanzlik and Spaulding, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 847; Gurchot, Hanzlik and Spaulding, *J. Pharm. Exp. Therap.*, 1931, **45**, 427.

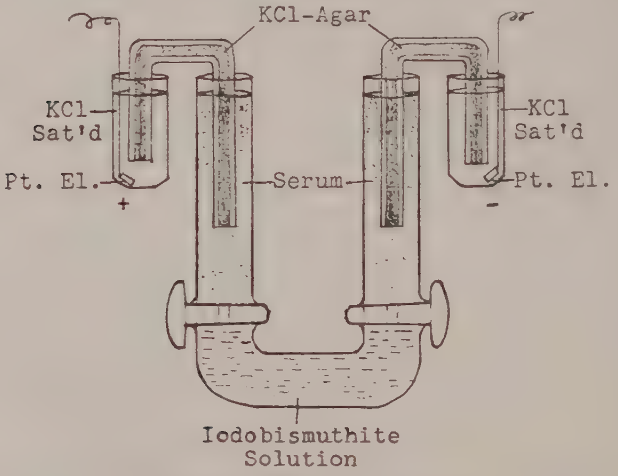

FIG. 1.
Diagram of arrangement for electromigration of iodobismitol in colloidal systems.

As the electrolytic cell used may be of general interest, and its application appears to be novel, a short description and a diagram are presented. The parts of the cell are sufficiently evident from the diagram in Fig. 1. We commonly use Pyrex glass tubing of 8 mm. bore for the U-tube and suitable stop-cocks, height over all being 12 cm., and the part under the stop-cocks, about 2 cm. The tubes holding the saturated KCl solution and the platinum electrodes are ordinary small test tubes, 70x14 mm. The glass tubes for the KCl-agar bridges are of 3 mm. bore, total length 22 cm. The agar-gel is saturated with KCl in the usual manner. The cell is operated as follows: The U-part beneath the stopcocks is filled with the solution to be migrated to the upper level of the stop-cocks, which are then closed; any solution in the chambers above is washed out and the serum, or other medium, is run in to the same level in both chambers. The KCl-agar bridges, connected with the saturated KCl solution, are now inserted into the chambers to equal positions about 4 cm. above the stop-cocks, which are then carefully opened, and a current of 110 v. is turned on.

When the ion is colored, as in the case of iodobismuthite, the boundary is seen to move toward the anode and correspondingly away from the cathode in less than 1 hour. A standard time of 1 or 2 hours, with this cell, will suffice for many experiments; in other cases, 24 hours or longer may be necessary or desirable. By the end of 8 to 24 hours, the boundary may have reached the KCl-agar bridge. In qualitative experiments, the presence of an ion in the chamber fluids and in the agar, such as bismuth, may be simply identified by the color or by adding hydrogen sulphide test solution, or by both these tests. In quantitative experiments, the chamber fluids and the bridge-agars are analyzed separately.

The principal variables affecting the electromigration are: fluctuations in electric current which affect the time; electrolyte concentration of the fluid to be migrated and of the chamber-fluids which affect the speed, the higher the concentration the greater the speed; diffusion from the boundaries which affects the ion-content of the chamber-fluids, this being negligible in short experiments but must be deducted from the total ion-content of each chamber in long experiments, such as 24 hours or more; physical disturbances in the ordinary laboratory which tend to vitiate results with the cell. The agar is, of course, not affected by diffusion or physical disturbances, and therefore, the use of KCl-agar bridges is a decided advantage. An even greater advantage of the agar-bridges over bare platinum electrodes is that the decomposition voltage of any compound can be neglected.

In actual experiments with sodium iodobismuthite, the following results were obtained: Using 6% sodium iodobismuthite in ethylene glycol, boundary movement of the red fluid (BiI_5 ion) toward the anode occurred in about 1 hour, more definitely in 2 hours, and reddish-yellow staining of the KCl-agar anode in 5 to 6 hours; presence of bismuth being confirmed by positive tests with H_2S. In 24 hours, the KCl-agar anode was deep yellow to brownish and, since tests for free iodine were negative, the coloring was due to the (BiI_5) ion. On the other hand, the boundary moved away from the cathode, the chamber-fluid remained colorless and the KCl-agar cathode was not stained and tests with H_2S were negative. Similar results were obtained with iodobismitol (12% NaI, and 6% Na_2BiI_5 in ethylene glycol). Thus the results confirmed the original claims for the electronegativity of Bi in iodobismuthite. The same qualitative results were obtained for ethylene glycol, glycol acidified with acetic acid, 25% acetic in water, alcohol acidified with acetic acid, 95% alcohol, and horse serum in the chambers.

Quantitative results in 15 experiments with natural and dialyzed serums in the chambers, and 6% iodobismuthite in glycol, were as follows: Runs of from 3 to 24 hours, median 24 hours, gave 0.04 mg. Bi, median (range, 0.002 to 0.43 mg.) in the KCl-agar anode and 0.001 mg. Bi, median (range, 0 to 0.02 mg.), the median difference in favor of anionic Bi being 0.031 mg., or a concentration of 1:64,000 in the agar-gel. There were 2 gm. of KCl-agar gel in each bridge. The serum of the anode chamber showed 0.17 mg. Bi, median (range, 0.03 to 0.4 mg.), and of the cathode chamber, 0.02 mg. Bi, median (range, trace to 0.6 mg.), the median difference in favor of anionic Bi being 0.13 mg. or a concentration of about 1:100,000 in the serum. No deduction for diffusion was necessary from the Bi of the KCl-agar electrodes, but from the chamber-fluids quantities of 0.003 mg. (median) were deducted, according to results of 24-hour runs in 12 experiments of the same kind except for passage of the electric current. In the short runs, as in the qualitative experiments, the results were unaffected by diffusion, but, in long runs, some hydrolysis of the (BiI_5) ion apparently occurred. Nevertheless, the preponderant migration of the iodobismuthite in the vast majority of experiments was as the electronegative (BiI_5) ion in both serum and agar-gel. The bismuth in these experiments was estimated according to a method previously described.[3]

Conclusions. A simple electrolytic cell which guards against decomposition of labile, complex ions, which is also suited to tests of electromigration in colloidal systems and tends to overcome objections to the use of bare platinum electrodes in ordinary cells, is described. Electronegative migration of the colored iodobismuthite (BiI_5) ion is demonstrable in different solvents, and in colloidal systems, such as serum and agar-gel, in confirmation of previous claims for electronegativity of this bismuth compound by other methods. The colloidal migration in the model used correlates with the results on absorption and cerebrospinal penetration of the iodobismuthite.

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[3] Hanzlik, Mehrtens, *et al., Arch. Derm. Syph.*, 1930, **22**, 483.

6646

Reliability of Spinal Fluid Analysis in the Diagnosis of Drunkenness.

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The medico-legal importance of establishing the state of inebriety of an individual has given rise to a great mass of research on the reliability of analysis of the various body fluids as an index of drunkenness. Those most commonly used are the blood, the urine, and the breath, but most authors mention that except for its impracticability the spinal fluid should yield the most reliable information.

Nicloux[1] found that in dogs about an hour after ingestion of a single dose of alcohol the values in the blood and spinal fluid were approximately equal. Schumm and Fleishmann[2] administered a constant dose of alcohol to a series of patients, puncturing each at a different period after ingestion, and so obtaining a composite curve of blood and spinal fluid alcohol. They found that during the first hour the blood alcohol rose more rapidly than did the spinal fluid alcohol, but that during the decline of the blood alcohol the spinal fluid alcohol surpassed it and remained at a higher level. Because of their use of single punctures on various patients some of their figures fall far out of line. Recently Abramson and Linde[3] repeated this work, using a dose of alcohol proportional to body weight, and leaving the lumbar needle in place during the 3 hours over which they collected their specimens. They showed that the alcohol content of the spinal fluid rises more slowly than that of the blood, and reaches its maximum later. This maximum is lower than that reached by the blood. During the period of decline the spinal fluid alcohol remains higher than the blood alcohol.

In support of the contention that the spinal fluid alcohol is the best index of intoxication, Gettler and Freireich[4] showed in a series of cases coming to autopsy that there was a more constant relation between the alcohol content of the brain and spinal fluid than between

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* Supported by a grant from the Rockefeller Fluid Research Fund of Stanford University Medical School.

[1] Nicloux, M., *Compt. rend. de la Soc. de Biol.*, 1900, **52**, 620.

[2] Schumm, O., and Fleishmann, R., *Deutsche Z. f. Neivenheilkunde*, 1913, **46**, 275.

[3] Abramson, L., and Linde, P., *Arch. Internat. de Pharmacodynamie et de Ther.*, 1930, **39**, 325.

[4] Gettler, A. O., and Freireich, A. W., *J. Biol. Chem.*, 1931, **92**, 199.

the brain and blood. Some of their values appear rather bizarre, probably being accounted for by the fact that the material was obtained post mortem.

If we subscribe to their theory, we must assume that a person does not become intoxicated for an hour or so after taking liquor, since the work first quoted shows a lag of that long in the rise of the spinal fluid alcohol. This does not agree with our experience, which includes some 50 cases of experimentally induced intoxication, both by the intravenous and the oral route. We therefore undertook to confirm or refute the theory.

We first determined the course of the lumbar fluid alcohol after a dose of 1 cc. per kilo, administered intravenously by the technic described by us previously.[5] The results (Fig. 1) confirm the work of the previous investigators. In none of 7 cases so treated did the spinal fluid alcohol reach the level of the blood alcohol in less than 80 minutes, yet our observation of these patients, as well as of many others after the same dose, showed them to be more intoxicated at the end of the injection, when the spinal fluid alcohol was negligible, than at any subsequent time. This would seem to show that at least during the first hour after imbibition the spinal fluid alcohol is a poor index of intoxication.

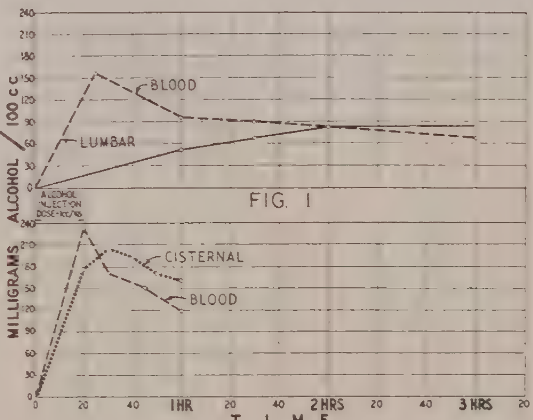

FIG. 1

FIG 2

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[5] Newman, H. W., and Mehrtens, H. G., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 145.

We repeated the experiment, but instead of removing the fluid by lumbar puncture as our predecessors had done we did cisternal punctures. The results (Fig. 2) demonstrated how promptly the values in the cisternal fluid follow those in the blood. The third figure shows a comparison of the rise in cisternal and lumbar fluid in another patient after a single injection. The lag of the lumbar fluid is striking.

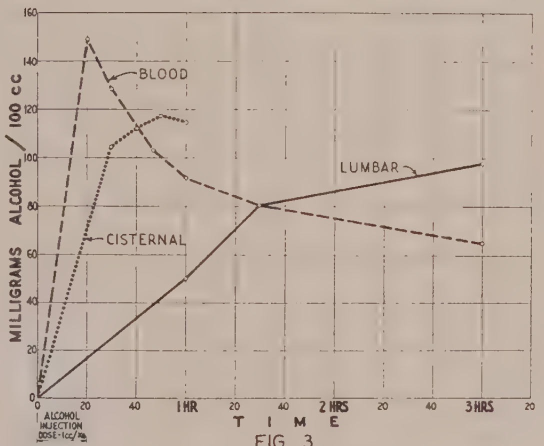

FIG. 3

Thus we can say that the alcohol content of the cerebral fluid closely follows that of the blood, and correlates well with the symptoms of intoxication, while that of the lumbar fluid shows a lag of about an hour. From this it would seem that at least during a period of an hour or so after the ingestion of alcohol the content of the cisternal fluid is a better index of intoxication than that of the lumbar fluid, while that of the blood is probably the best of all.

The explanation of the more rapid rise in the cisternal fluid over the lumbar fluid is still obscure. Two possibilities present themselves: that the alcohol is secreted by the choroid plexuses and diffuses slowly down the cord; or, that the process is a simple transudation from the blood vessels, the greater vascularity of the cerebral end of the system accounting for the more prompt attainment of equilibrium in that region. We intend to investigate this point in detail.

Missouri Section.

Washington University School of Medicine, February 8, 1933.

6647

Comparison of Properties of Individual Axons in the Frog.

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In large mixed nerves, such as the sciatic, the action potential started by local stimulation is much more protracted after conduction than at the locus of stimulation, and, in the former case, presents 3 principal elevations of the continuous potential.[1] Bishop and Heinbecker recognize 4 main elevations and believe that the potentials contributed to these elevations are supplied by fibers of different types.[2, 3] Measurements of the duration of axon potentials hitherto have been based on superimposed axon responses recorded at the site stimulated. Inasmuch as axons respond to stimulation with different latencies[4] this method cannot give the axon potential in its true form. By employing nerves containing only a few fibers at the lead, long distances of conduction and very high amplification, it is possible to record discretely the undistorted potentials of some, often of all, and to ascertain the characteristics of each, of the constituent axons.

When either the relative voltage of threshold induction shocks or the rheobasic voltage from a condenser is plotted against conduction rate the resulting curves both are hyperbolic in form, the slower fibers having the higher thresholds. Likewise a smooth curve, hyperbolic in form, can be drawn between the points relating time to maximum of the action potential to conduction rate; the slower the conduction rate the longer is the time to maximum. And in so far as it is possible to correlate the data on the relation of the absolutely refractory periods to conduction rates the result is a similar hyperbolic curve. The refractory periods without exception are longer than the corresponding times to maximum. The data on action voltage, due to possible occasional failure to recognize deflections produced by 2 perfectly superimposed axon potentials, are not absolutely reliable. The individual curves of correlation with conduction rate seem to deviate around a straight line inclined steeply downwards from the quicker to the slower axons. Since the slower fibers have the smaller diameters and since a larger fraction of the intrinsic potentials of the former must be shunted by inactive tissue, this result may not be inconsistent with the assumption[5] that the intrinsic potentials all are alike in amplitude.

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[1] Erlanger, J., and Gasser, H. S., *Am. J. Physiol.*, 1930, **92**, 43.

[2] Bishop, G. H., and Heinbecker, P., *Am. J. Physiol.*, 1930, **94**, 170.

[3] Heinbecker, P., and Bishop, G. H., *Am. J. Physiol.*, 1931, **96**, 613.

[4] Blair, E. A., and Erlanger, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 926.

Comparison of these several properties is complicated by the fact that they have some, seemingly spontaneous, variability. The points in every case distribute themselves somewhat irregularly about the plotted curves but the curves seem to be free of discontinuities in the range of fibers conducting faster than 2 m.p.s.; beyond that there still is some uncertainty. The results, therefore, fail to support the view that there are fiber types distinguishable by time to maximum, conduction rate, irritability and refractory period. Nevertheless, in keeping with our observations on large nerves we find that the conducted axon potentials tend to sum to form some 3 elevations corresponding in general with A, B, and C of the action potential of large nerves, but the relative positions of these elevations in the different nerves, particularly in skin nerves, is quite variable. Since it is possible to recognize differences in the physiological responses of individual axons it is no longer incumbent upon physiologists to adhere to the doctrine of specific nerve energies.

6648

True Polyvalence of Pure Bacteriophages.*

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As found in their natural environment, bacteriophages often exhibit lytic activity against a variety of organisms sometimes quite unrelated to each other. This polyvalence is usually due to the presence of several independent phages which can be separated from each other by special procedures. Frequently, however, even such isolated single phages, satisfying all known criteria of purity, may exhibit activity against several more or less closely related species of bacteria. In such cases the characteristics of these bacteriophages usually remain immutable no matter which one of the organisms susceptible to their activity is used for their propagation.[1] Since the active principle in such instances is regenerated equally well on either one of the several susceptible organisms, it is perhaps possible to suppose that the active principle is represented by a very similar if not an identical substance in each case, and that the bacteria in turn have a common element in their composition ("receptor") rendering them susceptible to it. If this could be proven, the nature of this true polyvalence of pure phages would be satisfactorily explained. We have recently made some observations which bring supporting evidence for such a view.

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[5] Gasser, H. S., and Erlanger, J., *Am. J. Physiol.*, 1927, **80**, 522.

* This work was supported from a grant by the Rockefeller Foundation to Washington University for Research in Science.

One of the coli-phages in our collection, used in the various experiments in our laboratory for nearly 6 years and invariably found to be pure according to all accepted criteria, exhibits the same degree of lytic activity against our type strains of *B. coli, B. dysenteriae* "Shiga", and *B. dysenteriae* "Flexner", irrespective of whether it is propagated with one or another of these organisms. When this phage is subjected to heating, all 3 valencies disappear at the same time. However, if the speed of inactivation by heat is delayed by the addition of glycerin or of saccharose,[2] the Shiga and Flexner valencies disappear slightly in advance of that against *B. coli* (see protocol). It is thus that after the exposure to heat at 70°C. for 30 minutes it is possible to recover a weak phage (titer 10^{-3} cc.) active against *B. coli* only.

If this monovalent phage is now introduced into the broth cultures of each of the organisms separately it is regenerated only in the presence of *B. coli* and not in the presence of *B. dysenteriae* "Shiga" or *B. dysenteriae* "Flexner". However, the phage after a single passage on *B. coli* is found to have recovered its power to cause lysis of *B. dysenteriae* "Shiga" and *B. dysenteriae* "Flexner" (titer 10^{-1} and 10^{-3} cc. respectively) and after a third consecutive passage *with B. coli* its lytic activity for *B. dysenteriae* "Shiga" and *B. dysenteriae* "Flexner" has been quantitatively restored (titer 10^{-9} cc.).

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[1] Bronfenbrenner, J., and Korb, C., *J. Exp. Med.*, 1925, **42**, 821.

[2] Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 802.

		(Control) Unheated								Mixtures heated, submerged in sealed tubes at 70°C. and tested at 10 min.								20 min.								30 min.							
		cc.				cc.				cc.				cc.				cc.				cc.				cc.				cc.			
P. C. phage (coli)		1				1				1				1				1				1				1				1			
Plain broth		2				—				2				—				2				—				2				—			
Sat. solution of sucrose in plain broth		—				2				—				2				—				2				—				2			
Organisms subjected to lysis	cc.	1*	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Extent of lysis observed (+ = lysis; — = no lysis)	10^{-1}	+	+	+	—	+	+	+	—	+	+	+	—	+	+	+	—	—	—	—	—	+	+	+	—	—	—	—	—	+	—	—	—
	10^{-2}	+	+	+	—	+	+	+	—	+	+	+	—	+	+	+	—	—	—	—	—	+	+	+	—	—	—	—	—	+	—	—	—
	10^{-3}	+	+	+	—	+	+	+	—	+	+	+	—	+	+	+	—					+	+	+	—					+	—	—	—
	10^{-4}	+	+	+	—	+	+	+	—	+	+	+	—	+	+	+	—					+	—	+	—					—	—	—	—
	10^{-5}	+	+	+	—	+	+	+	—	+	+	+	—	+	+	+	—					—	—	—	—								
	10^{-6}	+	+	+	—	+	+	+	—	+	—	—	—	+	+	+	—																
	10^{-7}	+	+	+	—	+	+	+	—	—	—	—	—	+	+	+	—																
	10^{-8}	+	+	+	—	+	+	+	—					+	+	+	—																
	10^{-9}	+	+	+	—	+	+	+	—					—	—	—	—																
	10^{-10}	—	—	—	—	—	—	—	—					—	—	—	—																

Bacteriophage after heating at 70°C. 30 minutes propagated on each of the organisms and the resulting filtrates tested for lytic activity against each of the organisms for 3 successive passages.

						Propagated with															
	Titer in cc.	(Control)				*B. coli* Tested for activity against				*B. dys.* "Shiga" Tested for activity against				*B. dys.* "Flexner" Tested for activity against				*B. dys.* "Hiss" Tested for activity against			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Heated (30 min.) Phage retested before propagation	10^{-1}	+	—	—	—																
	10^{-3}	+	—	—	—																
	10^{-6}	—	—	—	—																
	10^{-9}	—	—	—	—																
After first attempt at propagation	10^{-1}					+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
	10^{-3}					+	—	+	—												
	10^{-6}					+	—	—	—												
	10^{-9}					+	—	—	—												
After second attempt	10^{-1}					+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
	10^{-3}					+	+	+	—												
	10^{-6}					+	+	+	—												
	10^{-9}					+	—	—	—												
After third attempt	10^{-1}					+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
	10^{-3}					+	+	+	—												
	10^{-6}					+	+	+	—												
	10^{-9}					+	+	+	—												

* 1 = *B. coli*. 2 = *B. shiga*. 3 = *B. Flexner*. 4 = *B. Hiss*.

Entirely analogous results were obtained with a *B. typhosus* phage exhibiting a secondary valency for *B. dysenteriae* "Flexner" and with *Staphylococcus aureus* phage, exhibiting a secondary valency for *Staphylococcus albus.* On the other hand when a polyvalent phage was produced by deliberately mixing several independent and unrelated phages (such as anti-coli and anti-typhosus or anti-staphylococcus) and the mixture was subjected to heating, some of the valencies lost during the heating could not be regenerated by the passage of the residual phage with a susceptible organism.

These experiments show, therefore, that when true multiple valencies are present in pure phages they are determined by some sort of a specific relationship among the susceptible bacteria perhaps remotely analogous but not identical with the relationships determining serological group reactions.

Incidentally, these results offer additional evidence against the parasitic nature of phage—for if phage were a parasite capable of invading *B. coli, B. dysenteriae* "Shiga", and *B. dysenteriae* "Flexner", it is difficult to see how heat could destroy its power of invading the 2 latter organisms while leaving the invasive power for the first unimpaired.

Furthermore, the subsequent recovery of lytic power for *B. dysenteriae* "Shiga" and *B. dysenteriae* "Flexner" can not be explained on the basis of "adaptation" of the parasite, since in these experiments the development of additional valencies by the monovalent anti-coli-phage took place as a result of the passage with *B. coli* only, and did not take place when *B. dysenteriae* "Shiga" or *B. dysenteriae* "Flexner" were exposed to it.

6649

Stimulation of Bacterial Metabolism by Bacteriophage.*

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Examination of an automatic cinematographic record of the progress of lysis of *B. coli* in the presence of bacteriophage suggested that addition of phage to pure cultures of *B. coli* causes an increase

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* This work was supported from a grant of the Rockefeller Foundation to Washington University for Research in Science.

in the rate of cell division as compared with the rate of division maintained by the same cultures in the absence of bacteriophage.[1]

This growth stimulating effect of bacteriophage was again observed in a very striking way, when the phage and bacteria were brought together on solid medium under circumstances which precluded the free lysis of bacteria. Under these conditions the density of bacterial growth was markedly increased under the drops of bacteriophage.[2, 3] These and subsequent observations lead us to conclude that the effect exerted by phage is primarily that of acceleration of the metabolic activities of susceptible bacteria (including the increased rate of multiplication) and that actual lysis, while the most striking feature of the phenomenon, is in fact only secondary in importance and occurs only as a result of this change in the metabolic rate and the consequent osmotic changes in the cells taking place if free water happens to be available.[4, 5]

The increase in the rate of multiplication of bacteria in the presence of phage has been observed by many investigators. However, in most instances the evidence was more or less indirect. More recently there appeared two accounts of experiments in which the authors attempted to count bacteria directly and concluded that the addition of phage does not cause acceleration of growth.[6, 7]

In view of the manifest importance of this question in connection with the proper understanding of the mechanism of the transmissible lysis we decided to carry out further experiments.

Two flasks, each containing 200 cc. of broth (pH 7.4), received a suspension of bacteria so diluted that the initial count in the flask approximated 10^5 organisms per cc. To one of the flasks was added 2 cc. of active phage (the concentration of phage was varied in different experiments); the other flask serving as control received 2 cc. of the same phage inactivated by heat.

At intervals samples were removed from each flask and examined as follows: (a) A portion of the sample was introduced into a cup of a nephelometer and compared against an arbitrary turbidity standard. The observations were recorded in arbitrary units of the nephelometer scale.

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[1] Bronfenbrenner, J., Muckenfuss, R. S., and Hetler, D. M., *Am. J. Path.*, 1927, **3**, 562.

[2] Bronfenbrenner, J., and Hetler, D. M., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 480.

[3] Hetler, D. M., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 806.

[4] Bronfenbrenner, J., and Hetler, D. M., *Am. J. Path.*, 1928, **4**, 622.

[5] Bronfenbrenner, J., *J. Bact.*, 1930, **19**, 19.

[6] Eaton, M. D., *J. Bact.*, 1931, **21**, 143.

[7] Andrews, C. H., and Elfort, W. J., *Brit. J. Exp. Path.*, 1932, **13**, 13.

(b) A second portion of the sample was immediately transferred to a solid medium for the counting of the number of viable bacteria. The media used for this purpose were designed to arrest the progress of lysis. In some experiments 50% gelatine, in others 4% agar or a 2% agar containing 0.75% of sodium citrate, were used. (The latter was found less satisfactory.)

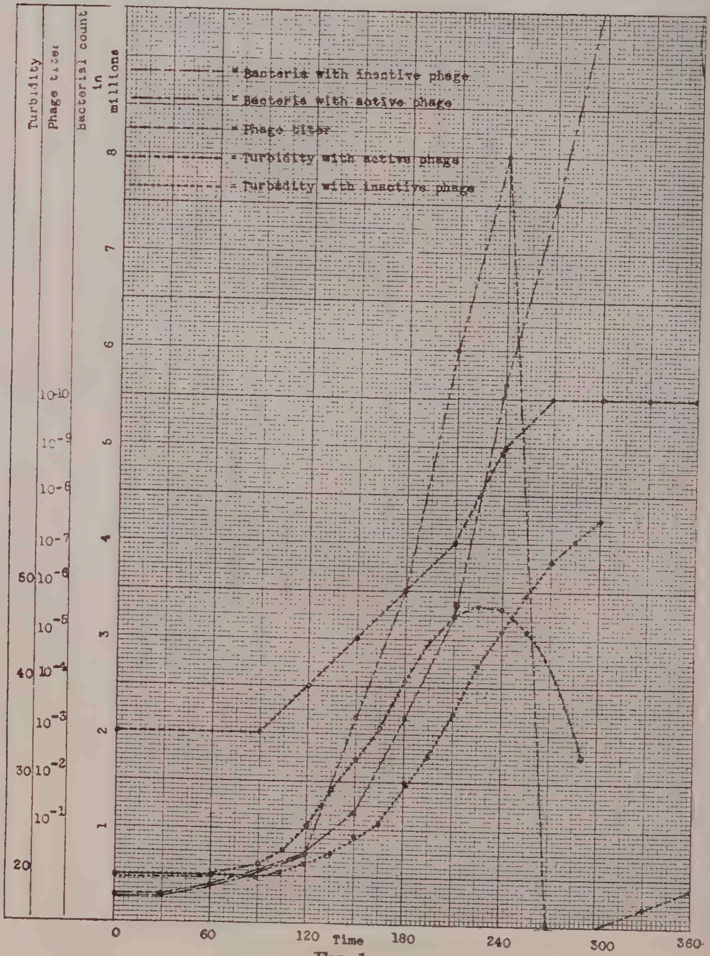

FIG. 1.

(c) A third portion of the sample was titrated for bacteriophage content.

As can be seen from a protocol of one of the experiments, the culture growing in the presence of active bacteriophage increases in turbidity faster than does the control culture. The curves representing the turbidity changes are in general similar to those representing the changes in bacterial count, except that acceleration of growth in the presence of phage as recorded by the increase in turbidity is apparent earlier than it could be detected by the bacterial count. This we believe is due to the fact that gelatine fails to completely stop all lysis and certain number of bacteria present in the samples undergo lysis after being transferred upon the plates. In spite of this inaccuracy of the method, the results show quite clearly that in the presence of phage the lag period is somewhat shortened, and that bacteria multiply at a greater rate than they do in the absence of phage. As the concentration of phages increases in the culture up to the critical titer (10^{-8} cc.) the massive lysis takes place.

These experiments substantiate by direct evidence our earlier observation that phage stimulates the metabolism of bacteria. In order to elicit this phenomenon, however, it is necessary to adjust the experiment in such a way that lysis of bacteria would be delayed, otherwise the lysis occurring simultaneously with growth of bacteria obscures the results. Essentially analogous results were obtained with *B. coli* and staphylococcus.

6650

Is Ant. Pituitary Hormone Demonstrable in Urine of Graves Disease, in Urine of Guinea Pigs Injected With Ant. Pituitary Extract?

MARGARET G. SMITH AND ELIZABETH MOORE. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine.

Loeb and Bassett[1] have shown that an acid extract of cattle anterior pituitary produces hypertrophy of the thyroid gland of guinea pigs. They noted that this experimentally produced hypertrophy characterized by an enlargement of the acinar cells, softening and absorption of colloid, and an irregular and often slit-like shape of the acini, and by the formation of papillary ingrowths into the acini resembles the pathological change in the thyroid gland in Graves disease. In a series of papers from this laboratory this similarity between the effects produced by the injection of extracts of the anterior pituitary gland of cattle and the symptoms of Graves disease has been further emphasized. Loeb and Friedman[2] have produced exophthalmos in guinea pigs by repeated intraperitoneal injections of acid extract of the anterior pituitary. Siebert[3] showed that this extract produced a rise in metabolism which was counteracted by the administration of potassium iodide, observations which accorded with the depressing effect of potassium iodide on the hypertrophy of the thyroid gland elicited by anterior pituitary which had previously been noted by Silberberg.[4] McCordock and Hageman[5] produced tachycardia in guinea pigs by means of this extract. Closs, Loeb, and MacKay[6] in joint investigations from this laboratory and the Scripps Metabolic Clinic in La Jolla, found the changes in the distribution of iodine in the thyroid gland and in the blood called forth by extract of anterior pituitary to be the same as those observed in Graves disease. Notwithstanding the similarity between these two conditions, Loeb[7] believes that while the anterior pituitary may possibly play a certain rôle in the etiology of Graves disease, there is reason for assuming that various other factors may elicit an overaction of the thyroid gland.

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[1] Loeb, Leo, and Bassett, R., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.

With the increased pituitary function occurring during pregnancy an excretion of large amounts of pituitary substance in the urine has been demonstrated by Ascheim and Zondek.[8] However, Loeb[9] has shown that this substance, present in the urine of pregnant women, has no effect on the thyroid gland of guinea pigs. In view of the presence of large amounts of pituitary substance in the urine in association with the increased pituitary function of pregnancy, and considering the similarity which exists between the symptoms of Graves disease and the effects of injections of anterior pituitary extracts, it was of interest to determine whether it would be possible to demonstrate the presence of an anterior pituitary substance in the urine of patients with Graves disease. We studied, therefore, the effect of injections of urine of patients showing marked symptoms of Graves disease on the thyroid gland of guinea pigs. Especially did we wish to determine whether such injections would produce hypertrophy of the thyroid gland.

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[2] Loeb, Leo, and Friedman, Hilda, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 648.

[3] Siebert, Walter J., and Thurston, E. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 652.

[4] Silberberg, Martin, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 166.

[5] McCordock, H. A., and Hageman, P., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 297.

[6] Closs, Karl, Loeb, Leo, and MacKay, Eaton, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 170.

[7] Loeb, Leo, *Klin. Woch.*, 1932, **51**, 1.

[8] Ascheim and Zondek, B., *Klin. Woch.*, 1927, **28**; 1928, **30**, **31**.

[9] Loeb, Leo, *Endocrinol.*, 1932, **16**, 129.

Urine was obtained from 4 such patients before any treatment was instituted and, in addition, from 3 patients in which the exophthalmos persisted several months after thyroidectomy. The urine from each of the 4 untreated patients was injected into at least 2 guinea pigs weighing between 170 and 190 gm. A daily intraperitoneal injection of 3 cc. was given on 10 consecutive days and the animals were killed on the eleventh day. In 3 instances, one additional animal received heated urine. The 3 specimens of urine, specimens II, IV, and V, obtained from patients several months after thyroidectomy were injected into 4, one, and 2 animals respectively. Of the 4 animals receiving specimen II, 2 received 5 intraperitoneal injections of 3 cc. each of unheated urine, and 2 others received 9 similar injections of heated urine. The animals receiving specimens IV and V were given 10 intraperitoneal injections of 3 cc. of unheated urine on consecutive days.

Results. In no instance did the thyroid of the injected guinea pigs show any hypertrophy. We may then conclude that under the conditions of our experiments it has not been possible to demonstrate the presence, in the urine of patients suffering from Graves disease, of a substance stimulating the thyroid gland to active growth. And inasmuch as it has been shown that a correspondence exists between the structure of the thyroid gland and its function, we may furthermore conclude that it has not been possible to demonstrate in the urine of patients suffering from Graves disease a substance stimulating the thyroid gland to increased function.

The question arises as to whether the lack of such a substance in the urine in Graves disease permits the conclusion that such a substance is not present in the body fluids of patients affected by this disease. It is conceivable that such a substance even were it present in the blood might not be excreted in the urine. We therefore carried out several series of experiments in which we tested the degree to which extract of anterior pituitary gland previously injected into the guinea pig is excreted in the urine as well as the rate at which it is excreted. Five series of 2 animals each were injected intraperitoneally with 3 cc. of acid extract of cattle anterior pituitary daily for five days. About 4 hours following the last injection the animals were killed and the urine collected directly from the bladder. Each day for 5 consecutive days the urine thus obtained, which averaged a little less than 2 cc. per day, was injected into a guinea pig weighing 190 gm. The animal was killed on the sixth day. The characteristic change in the thyroid gland produced by acid extract of anterior pituitary could not be demonstrated in this animal. We repeated this experiment and again obtained negative results.

These experiments suggested that acid extract of anterior pituitary gland of cattle when injected intraperitoneally in guinea pigs is either not at all, or to only a very slight extent, excreted by way of the kidney. However, we tested these conclusions as follows: Instead of a series of injections of 3 cc. of extract of anterior pituitary, we gave one huge intraperitoneal injection of 4 cc. to each of 6 male guinea pigs, each animal weighing approximately 180 gm. This represents a very large dose, larger than any given previously in this laboratory in a single injection. At intervals of 3 and 7 hours following the injection the urine was collected by exerting pressure over the bladder. On the two days following the injection, the urine was collected 3 times at intervals of from 3 to 3½ hours. Three guinea pigs, weighing from 180 to 190 gm., received this urine. One animal was injected intraperitoneally for 5 days with the urine collected on the first day. A second and third guinea pig were similarly injected with the urine obtained on the second and third day respectively. The animals were killed on the sixth day following the beginning of the injections. Our results were as follows: the thyroid of the guinea pig receiving the urine from the first day's collection showed a slight degree of hypertrophy. In many parts of the gland, the epithelium of the acini was somewhat increased in height. The colloid in many acini was somewhat softened as indicated by a paler color and by an increased size of peripheral vacuoles. In the center of the gland, we found a few acini converted into slits. However, in some parts of the gland, hardly any hypertrophy was noticeable. The acini were of normal size and the colloid hard. There was, therefore, in this case a definite although a moderate hypertrophy found. In the animal receiving the urine from the second day there was hardly any change, while in the animal receiving the urine from the third day's collection completely negative results were obtained.

This experiment was repeated. Each of 16 male guinea pigs was given a single intraperitoneal injection of 4 cc. of acid extract of cattle anterior pituitary and the urine collected for 3 days in the same way as in the preceding experiment. Six female guinea pigs, ranging in weight between 180 and 195 gm., were injected intraperitoneally for 5 consecutive days; 2 receiving the urine from the first day's collection; 2, the urine from the second day's collection; and 2, the urine from the third day's collection. No definite hypertrophy in the thyroids of these 6 animals resulted from these injections.

We may therefore conclude that even when present in the body in large amounts at least only a very small fraction of the hormone contained in the acid extract of cattle anterior pituitary which causes hypertrophy of the thyroid gland is excreted in the urine of guinea pigs. Perhaps this hormone, in contrast to the pituitary-like hormone of pregnant women, which is excreted in the urine in large amounts, is bound to such organs as the thyroid and ovary and then changed in some way before its excretion. The failure to demonstrate such a substance in the urine of patients suffering from Graves disease is, therefore, not inconsistent with a possible increase of pituitary function in this disease.

6651

Distribution of Vagus and Sacral Nerves to the Large Intestine.

CHARLES A. SCHMIDT. (Introduced by A. Kuntz.)

From the Department of Micro-Anatomy, St. Louis University School of Medicine.

There still remains considerable disagreement regarding the distribution of vagus and sacral nerves to the large intestine. According to Klee[1] vagus fibers do not reach the large intestine. Carlson[2] has recently reported experiments in which stimulation of the sacral nerves in the dog caused contraction of the circular and longitudinal muscle of the entire colon.

The present experimental anatomical investigation was carried out on cats and dogs. The vagus and sacral nerves respectively were sectioned in 2 groups of animals. Three weeks after operation the animals were killed and the large intestines were removed and studied both macro- and microscopically. Pyridine silver sections were made from 3 levels; viz., the ascending colon, the distal half of the transverse colon, and the middle portion of the descending colon.

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[1] Klee, P., *P. A.*, **145**, 594.

[2] Carlson, J. A., *J. Am. Med. Assn.*, **94**, 78.

Pyridine silver sections from the corresponding levels of the normally innervated large intestine reveal longitudinal nerve fiber bundles in the serous layer, which increase in size and number toward the distal end.

Pyridine silver sections from the large intestine of animals in which the vagi were sectioned just below the diaphragm showed degenerated nerve fiber bundles in the ascending and transverse portions of the colon. These obviously are vagus fibers. They are less numerous in the transverse than in the ascending colon. No evidence of degenerated nerve fibers was observed in sections of the descending colon. These results indicate that vagus fibers are distributed to the large intestine at least as far as the distal portion of the transverse colon.

A study of the pyridine silver sections from the same levels of the large intestine of animals in which all the sacral nerves were sectioned, revealed degenerative changes throughout the entire large intestine. These changes were less marked in the sections of the ascending and transverse portions of the colon than in those of the descending colon. In the latter, all the fibers in the bundles which traverse the serosa had undergone degeneration. The fibers of the myenteric plexus also were considerably reduced. The presence of degenerated nerve fiber bundles throughout the entire large intestine obviously indicates that sacral fibers are distributed to the entire colon. These fibers, however, become progressively fewer from the distal end of the transverse colon towards the proximal end of the ascending colon. These experimental microanatomical findings are in full accord with the physiological findings reported by Carlson.

Macroscopic study of the large intestine deprived of its sacral nerves suggests a marked diminution in the tonus, particularly of the descending colon.

6652

The Two Main Types of Anterior Pituitary Gland Present in Different Species of Animals.*

LEO LOEB AND HILDA FRIEDMAN.

From the Department of Pathology, Washington University School of Medicine.

We have shown[1, 2, 3] that the anterior pituitary glands of various species of animals differ in their effects on the thyroid gland and on the sex organs. In accordance with the character and intensity of these effects, we could arrange the various anterior pituitaries in a definite order. We also included urine of pregnant women and assigned to it a place between cattle anterior pituitary, and rabbit and cat anterior pituitary. The differences between rat, rabbit, cat, and guinea pig anterior pituitaries depended only on differences in the quantities of the active substances present, which were especially pronounced, if we compared the anterior pituitary of the guinea pig with the anterior pituitaries of the other animals, but it was impossible to equalize by variations in the quantities of substance used the effects exerted by cattle anterior pituitary and by urine of pregnant women with the effects exerted by the anterior pituitaries of the 3 species mentioned.

We now have examined also the effects of the anterior pituitary glands of sheep and hog. We prepared extracts from these glands in exactly the same manner as in the case of cattle gland: 5 gm. of dried and powdered gland were extracted with 100 cc. of dilute acid during a period of 2 days. The final extracts were then made very slightly alkaline and filtered through a Seitz filter.

In additional experiments, we extracted the gland tissue directly with 0.9% NaCl solution for 2 hours and extracts obtained were filtered through Seitz filters. These latter extracts showed only very weak effects, owing to the short time of extraction.

The effects of these extracts were tested on 24 guinea pigs, each weighing about 190 gm. Daily injections were made during periods varying between 5 and 10 days. The ovaries were studied in serial sections, the other organs (uterus, cervix, vagina), thyroid gland and, in some cases, other organs were examined microscopically in the usual way, but not in serial sections.

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* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 642.

[2] Loeb, Leo, *Endocrinol.*, 1932, **16**, 129.

[3] Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1128.

The results were as follows: The regular extracts of anterior pituitary glands of sheep and hog behaved approximately in the same way as did the corresponding extracts of anterior pituitary of cattle. In the thyroid gland they produced a marked hypertrophy and hyperplasia of the acinus cells associated with irregularity in the shape of the acini and a very far going solution and absorption of the colloid. These effects were the more marked, the greater the quantity of the extract that was injected. With the very weak extracts these effects would be lacking or almost lacking.

The ovaries all underwent a very pronounced atresia of follicles, only very small follicles remaining preserved. Thus a hypotypical condition of the ovaries resulted. This may have been partly due to or accentuated by the loss in weight or the lack of a more considerable gain in weight of these guinea pigs during the period of injection. But it was also due, in all probability, to a direct injurious effect which these extracts exerted on the ovaries or to an absence of the normal growth-promoting and of the maturation-producing effect of the anterior pituitaries as found in other species. There was also a tendency to the production of usually small pseudo-corpora lutea, which owed their origin in the main to hypertrophic changes in the connective tissue elements and in the theca interna during the later processes of atresia, although we cannot exclude the possibility that in some cases remnants of the granulosa may have participated in these formations. Furthermore, the theca interna of quite atretic follicles often underwent a slight increase in size and thus produced a very rudimentary so-called interstitial gland; likewise connective tissue cells situated in the medulla of the ovary, especially in the neighborhood of the blood vessels, might show a certain hypertrophy, so that isolated strands of this so-called interstitial gland were produced. There was no very definite relation between the quantities of extract injected and the intensity of the changes produced in the ovaries comparable to what we had found in the case of the thyroid gland. The occurrence of these ovarian changes, except the hypotypical conditions which took place in all cases, was irregular. In some cases all these changes were found, in others one or the other alteration was lacking and in other animals all of these were absent.

The vagina was usually in an atrophic condition; there was no sign of increase in size of the epithelial cells, nor any proliferation of the lower cuboidal layer. The uterus did not usually show an active condition; in some cases it was relatively thin. However, occasionally a certain proliferative activity was noticed in the connective tissue of the uterine mucosa and in the glands, perhaps due to the presence of small pseudo-corpora lutea or of interstitial gland in the ovaries. The mammary gland was not proliferating, but in certain of the acini there was occasionally some secretory activity noticeable. In one case a piece of sheep anterior pituitary gland, the size of a rabbit anterior pituitary, was inoculated subcutaneously into a guinea pig daily for 7 days. Hypertrophy of the thyroid gland was lacking. The ovary showed normally developed follicles; there was no hypotypical condition, but a pseudo-corpus luteum containing an ovum had formed. The uterus did not show any special reaction. The mammary gland was nonproliferating with some indication of secretion.

We see then that anterior pituitary glands of cattle, sheep and hog exert approximately the same effects on the thyroid gland and sex organs of the guinea pig. In contrast with these species, the pituitary glands of rabbits, cat, rat and guinea pig allow the follicles to grow to full size and bring about maturation of the granulosa. They may cause even premature maturation of follicles which have not yet reached full size. They also give rise to the production of pseudo-lutein bodies in which the luteinized granulosa and theca interna are invaded by connective tissue and blood vessels, a change that represents a modified, hypertrophic type of atresia. These anterior pituitaries lead to the production of much interstitial gland, at the expense (1) of the theca interna of the follicles in the late stage of atresia and (2) of the connective tissue in the medulla of the ovary. The amount of interstitial gland and the size of the individual cells in this formation exceeds by far the corresponding formation produced by the anterior pituitaries of the first group of animals. Moreover, the effects are attained much more regularly by these latter anterior pituitaries. Comparing the effects of equal weights of anterior pituitary in the first and second group of species, the effects in the thyroid gland are more pronounced in the second group. The vagina shows usually only a partial proliferation in the second group except in the cases in which ovulation occurs, when there is a complete keratinization. The mammary gland is usually proliferating under the influence of the second group of anterior pituitary glands. We have previously discussed marked quantitative differences between the effects of the different species constituting the second group, for example: Inoculation of one anterior pituitary of the guinea pig repeated daily during a period of from 5 to 10 days causes only maturation of follicles and a slight production of interstitial gland in the medulla. It has no effect on the thyroid gland.

Only daily inoculation of from 5 to 6 anterior pituitaries leads to changes similar to those produced by one-half or of a whole anterior pituitary of rabbit or of one anterior pituitary of the rat; but the latter exercise usually a stronger effect on the thyroid gland. As stated previously, the marked production of pseudolutein bodies and interstitial gland seems to inhibit a full proliferation of the vagina and inhibits perhaps also ovulation.

In contrast to the marked quantitative differences between the anterior pituitaries of the different species of the second group, equal weights of anterior pituitary of the first group seem to exert about the same effects, although it is possible that the anterior pituitary substance of the hog is slightly more effective than that of the sheep, if equal weights of both pituitaries are compared. In this connection, it may be of interest to compare the weights of the anterior pituitary glands of some of the species which we have considered in these investigations:

Average weights of the anterior pituitary glands of different species: †Cattle: 1115 mg. Sheep: 350 mg. Hog: 125 mg. Male rabbit (weighing 1300 to 1800 gm.): 12 mg. Male guinea pig (weighing 400-500 gm.): 7.5 mg. Male rat (weighing 120 gm.): 3 mg.

Summary. Different species of animals can be separated into 2 classes in accordance with the effects which their anterior pituitary glands exert on the thyroid gland and sex organs of the guinea pig, namely, Class I: Cattle, sheep, hog. Class II: Rabbit, rat, cat, guinea pig. The effects of the anterior pituitary glands of these two classes of animals on the thyroid gland and sex organs of the guinea pig are compared. We intend to investigate the possible effect of variations in the age of the donors of the anterior pituitary glands.

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† The age of the cattle used in these experiments was on the average 3 yrs., while that of the sheep and hogs was 10 months.

Minnesota Section.

University of Minnesota, February 15, 1933.

6653

Effect of Castration on Hypophysis Cerebri of the Male Albino Rat.

SAM I. STEIN. (Introduced by A. T. Rasmussen.)

From the Department of Anatomy, University of Minnesota.

In agreement with other authors, it was found that the hypophysis of the male albino rat increased in size as a result of castration.

As seen in the table, by means of the paper weight method on serial sections, it was found that this increase in size is due solely to an increase in size of the pars anterior. Pars posterior and pars in-

TABLE I.

Group	No. animals	Av. age	Av. body wt.	Av. body length	Av. wt. whole gland	Pars Anterior		Pars Posterior		Pars Intermedia	
		days	gm.	cm.		wt.	%	wt.	%	wt.	%
I. Controls	8	88.5	251	21.5	7.4	6.09	82.25	.79	10.92	.50	6.82
II. ”	8	109.0	254	22.0	8.6	7.08	82.36	.95	11.06	.57	6.58
Aver.		98.7	252	21.8	8.0	6.59	82.30	.87	10.99	.54	6.70
III. Test	9	88.8	202	20.3	10.9	9.42	86.72	.83	7.70	.46	4.18
IV. ”	11	106.0	215	20.9	12.3	10.77	87.53	.88	7.26	.55	4.52
V. ”	7	106.0	223	21.3	13.2	11.52	87.34	.93	7.17	.54	4.16
Aver.		100.0	213	20.8	12.0	10.52	87.21	.88	7.38	.52	4.31

Explanation of grouping:

Group I—Control, littermates of Group III-Test, were killed at the same average age. These glands were preserved in a mixture of 10% formalin and saturated mercuric chloride. Had a control operation.

Group II—Control, littermates of Group IV and V Tests, were killed at the same average age. This material was fixed in Zenker-formol solution. Had a control operation.

Group III—Test—Castrated approximately 68 days. Same fixative as Group I Control. The epididymis was removed with the testis.

Group IV—Test—Castrated approximately 88 days. Same fixative as Group II Control. The epididymis was removed with the testis.

Group V—Test—Castrated at approximately 88 days. Same fixative as Group II Control. The epididymis was left intact.

termedia are practically identical in absolute weight in the control and test animals.

Large, vacuolated basophilic cells typical of those described as "castration cells" or "signet ring cells" were found in the glands of the test animals.

Allowing the epididymis to remain intact did not alter the results from total castration, either volumetrically or histologically.

A significant decrease in the body weight, and body length of the castrated animals was also found.

6654

Effect of Insulin on Blood Sugar of Rabbits During Infection.

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From the Department of Medicine, University Hospital, University of Minnesota Medical School.

It is generally believed that the effect of insulin is diminished during infection in diabetes patients, and that temporarily more must be given to maintain a normal blood sugar level. To obtain information on the subject the following experiments were performed:

Healthy rabbits were injected intravenously with 0.5 unit of insulin per kg. of body weight. The blood sugar was determined by the Folin-Wu-Benedict method before insulin injection and thereafter at 15 to 30 minute intervals for 3½ hours. After at least 2 curves were obtained on successive days, the rabbits were inoculated intracutaneously with 0.1 cc. of a 1-400 dilution of a culture of Type I pneumococci. Usually on the next day, when the tem-

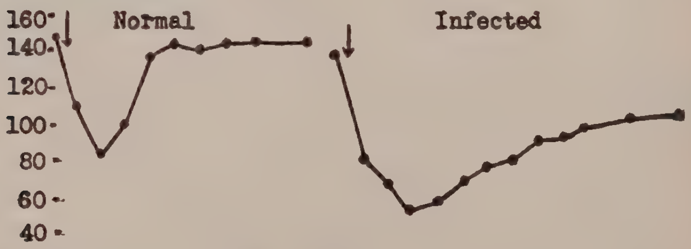

FIG. 1.

Blood sugar levels at 15 minute intervals after the injection of insulin into a normal rabbit and after it had been infected with pneumococci. Arrows indicate the time of insulin injection.

perature and local reaction indicated the development of infection, the insulin injection and blood sugar determinations were repeated. Tests were made daily, usually for 2 or 3 days until the animal died or recovered.

Results. In general, similar results were obtained in all 6 animals. A typical experiment is illustrated in the text-figure. It appears that insulin administered during infection takes a little longer to produce its maximum effect, but the effect is more pronounced and lasts considerably longer. It also appears that the effect on reducing the blood sugar is in proportion to the gravity of the infection. In one animal, after insulin injection on the second day of infection the blood sugar dropped progressively to 78 mg. in 1¼ hours when death occurred. In another (temp. 41°C.) the level dropped even more rapidly. Convulsions occurred when the level dropped below 50 mg. and death followed about 30 minutes later with a level of 22 mg.

6655

Presence of Clostridium botulinum in Livers of Birds not Affected with Botulism.

M. F. GUNDERSON. (Introduced by R. G. Green.)

From the Department of Bacteriology and Immunology, University of Minnesota, the Minnesota State Department of Conservation, and the Disease Investigations Project, Bureau of Biological Survey, Department of Agriculture, Washington, D. C.

There has been some difference of opinion as to whether or not *Clostridium botulinum* possesses the ability to invade tissue. The identification of western duck sickness with botulism[1, 2] gives to this problem considerable hygienic importance, and the morbidity during an epizootic furnishes a sufficient number of birds to subject this problem to statistical analysis.

It has been observed[3] that cultures made from animals dying of lamsiekte contained botulinum in 50% of the cases, while 3% of the animals dying from other causes yielded such positives. During experiments by others regarding the ability of botulinum to pro-

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[1] Kalmbach, E. R., *Science*, 1930, **72**, 658.

[2] Kalmbach, E. R., *Science*, 1932, **75**, 57.

[3] Scheuber, J. R., 15th An. Rep., S. A. Director of Veterinary Research, 1929, Part I, 223.

duce infection, some evidence indicates that even the detoxified spores can pass from the intestinal tract to the liver. However, the opinion has been expressed that *Cl. botulinum* does not usually invade the body, but that it may occasionally be recovered from the liver or spleen. In a discussion which also presents the literature,[4] experiments in which detoxified spores were fed and consequent deaths recorded are questioned on the basis that it is extremely difficult to free the spores completely from all traces of toxin. We believe that positive liver cultures produced by the ingestion of detoxified spores may be explained as due to the presence of minute traces of toxin. We have been unable to produce death in pigeons or ducks even with massive doses of detoxified viable spores.

Late in the fall of 1931, 34 ducks were examined after a lead-poisoning outbreak. Since the weather was cold, the dead birds were received in a frozen condition. The live birds were killed and autopsied immediately, the livers being removed aseptically. Toxicity tests and typing experiments showed *Cl. botulinum* Type C to be present in one of the liver cultures, while Type A was found in 2 cases, one of which was a duck received alive and killed immediately before necropsy.

During the fall of 1932, 2 botulinum cultures were obtained from a group of 5 ducks received from the Minnesota State Conservation Department. One culture was Type B; the other contained Types A and C.

Thirty-one livers were cultured from normal grouse, that had been shot, but the shot had not penetrated the viscera. One culture was positive for *Cl. botulinum,* Type A.

These results indicate that birds can harbor the botulinum organism, the presence of which possibly represents a previous exposure to toxic material from which the bird recovered. The difference between grouse and ducks probably represents variations in the toxin hazards of their respective environments.

Aside from the possibility that the presence of *Cl. botulinum* in such birds constitutes a potential danger in game birds as human food, if handled carelessly, the fact that such botulinum "carriers" provide a means for the dissemination of the organism is of greater importance. Migratory birds feeding in enzootic duck-sickness areas could thus increase the amount of soil infection in other favorable areas if they should die elsewhere and release *Cl. botulinum,* Type C, from their bodies. Types A and B could be carried likewise. In birds cultured from Minnesota, Type A is most frequently encountered, although in the case of the ducks it can not be stated with certainty that the organism recovered was picked up in Minnesota. The presence of *Cl. botulinum* in the livers of various members of the wild-life population of an area would constitute a valuable criterion as to the presence of *Cl. botulinum* in soil. The types of botulinum recovered from the livers of normal ducks representing the principal migratory routes followed in the United States would yield data of significance as to the botulinum infestation in the 3 areas.

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[4] **Topley and Wilson, The Principles of Bacteriology and Immunology. William Wood and Co., New York. 1929.**

The results obtained from the cultures just discussed will be compared with data obtained by use of the pigeon as the experimental subject. Such data make up a portion of a more complete study to be presented elsewhere.[5]

The occurrence of *Cl. botulinum* in the livers of normal pigeons was investigated. Twenty pigeons were killed and their livers removed aseptically for culture. Injections to determine toxicity yielded negative results. The failure to find organisms in the livers of normal pigeons from this region is not surprising, since *Cl. botulinum* is not a common soil inhabitant here, and the food habits of pigeons render them less likely to come in contact with grossly contaminated food than is the case with ducks.

One cubic centimeter of Type C culture, detoxified by heating at 80°C. for 30 minutes, was fed to each of 6 birds. A culture made from such detoxified spores was toxic upon injection after a period of incubation, thus indicating that the spores were viable. Forty-eight hours after spore ingestion, the birds were decapitated and liver cultures were made. *Cl. botulinum,* Type C, was present in one of the cultures.

These results verify those obtained from cultures of livers from natural sources. *Cl. botulinum* may be present in the livers of birds neither sick nor dead of botulism, and negative results may be obtained from cultures of birds dying of botulism.

Evidence which it may be possible to substantiate indicates that time is required for the organism to pass through the intestinal tract and reach the liver. In 2 of 3 cases of an intoxication of 9 hours' or less duration with Type C cultures, no organisms were detected in liver cultures. This may indicate that, in the case of a disease with a rapidly fatal termination, negative cultures may be expected.

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[5] Kalmbach, E. R., and Gunderson, M. F. Unpublished data in the Bureau of Biological Survey on "Western Duck Sickness; a Form of Botulism." (Being prepared for publication by the U. S. Department of Agriculture.)

TABLE I.

Type of botulinum recovered from liver cultures made from birds fed 1 cc. of Type A or C culture; effect of such feeding upon the birds. The dosage lethal for pigeons by injection was 0.001 cc. for the Type C, and 0.0001 cc. for the Type A culture.

Bird No.	Duration of disease	Results of typing experiments on liver cultures
	Birds fed Type C	
191	8 hr.	Negative
192	36 hr.	Type C
193	12 hr.	" "
194	9 hr.	Negative
195	12 hr.	Type C
196	Unaffected; killed after 144 hr.	" "
197	8 hr.	" "
198	18 hr.	" "
199	Lethargic; killed after 144 hr.	" "
200	Unaffected, killed after 144 hr.	" "
	Birds fed Type A	
578	12 hr.	Negative
579	12 hr.	Type A
580	30 hr.	" "
581	Unaffected; killed after 144 hr.	Negative
582	24 hr.	Type A
583	Unaffected; killed after 144 hr.	Negative
584	Unaffected; killed after 144 hr.	Type A
585	48 hr.	Negative
586	Unaffected; killed after 144 hr.	"
587	Unaffected; killed after 144 hr.	"

It is also postulated that passage or non-passage of the organism from the lumen of the intestine to the liver is dependent upon some factor that alters the permeability of the intestinal tract. Whether any intestinal disturbance coincident with the ingestion of detoxified spores would cause the appearance of organisms in the liver or whether the botulinum toxin is the controlling factor is also under investigation.

6656

Modification of Garrod's Method for Preparation of Homogentisic Acid from Urine.

GRACE MEDES.

From the Department of Medicine, University Hospital, University of Minnesota Medical School.

The basis of the methods now in use for the preparation of homogentisic acid from urine is that described by Garrod.[1] The urine is heated to boiling and 5 to 6 gm. neutral lead acetate are added for each 100 cc. of urine. As soon as the acetate is dissolved, the bulky gray precipitate which forms is removed by filtration and the filtrate is allowed to stand in a cool place, during which period the lead homogentisate deposits as acidular crystals. Garrod discusses the possibility of increasing the yield by the use of basic instead of neutral lead acetate. In the two cases studied by him the use of basic lead acetate did not increase the yield.

In the following modification of his procedure the pH of the urine is adjusted to the point at which lead homogentisate just fails to precipitate from the solution: Neutral lead acetate is added to the boiling urine, the precipitate filtered and the pH of the hot filtrate adjusted to about 5.6 with NH_4OH. At this pH the filtrate becomes faintly cloudy and on cooling all of the lead homogentisate crystallizes out. The lead salt is recrystallized once from hot water in the presence of an excess of lead acetate and with the pH again adjusted to 5.6. The free homogentisic acid is prepared by decomposing the lead salt with H_2S, concentrating the solution to a thick syrup under reduced pressure in an atmosphere of hydrogen, and saturating the concentrated solution with SO_2. On cooling the homogentisic acid rapidly crystallizes out.

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[1] Garrod, A. E., *J. Physiol.*, 1899, **23**, 512.

New York Meeting.

New York Academy of Medicine, March 15, 1933.

6657

Induced Ovulation in Amphibians by Injection of Antuitrin-S.

ALBERT E. OGILVIE. (Introduced by A. J. Goldforb.)

From the Department of Zoology, Columbia University.

Japanese newts, *Triturus (Molge) pyrrhogaster,* were given injections of Antuitrin-S, a sex-stimulating extract from the urine of pregnant women, twice daily for 5 days, with the result that eggs were laid on the sixth day. The total number of eggs laid was about 50 of which 27 were fertile and from which number 21 developed into swimming larvae. Both the males and the females were injected at the same time, 0.2 cc. twice a day, subcutaneously in the abdominal region.

The same extract was used in experiments on the Mexican axolotl, generally considered a neotenous form of *Amblystoma tigrinum,* with the following results.

Experiment I. 2 males and 2 females were injected during the same period, with 0.3 cc. of Antuitrin-S 3 times a day for 4 days. Several hundred eggs were laid on the fifth, sixth, and seventh days. These eggs were not fertile.

Experiment II. 2 males only were injected with 0.3 cc. three times a day, after which they deposited 15-20 spermatophores on the fourth day. 2 females were then injected (on the fourth day of the experiment) 3 times with 0.3 cc. and eggs were laid that night and on the succeeding 4 days. These eggs were mostly infertile, only a few of them going through the second cleavage stage.

Experiment III. 2 males were injected with the same amounts as in previous experiments. Twenty to 25 spermatophores were deposited on the fourth and fifth days. Two days later (on the sixth day) the females laid several hundred eggs, all of which were fertile.

From the last 3 experiments it will be seen that treatment of the male will arouse sexual activity resulting in the deposition of spermatorphores. These spermatophores are picked up by the females which lay their eggs without treatment.

The extract used in these experiments was obtained through the courtesy of Parke Davis and Company. The strength of the extract was 175 rat units per cc.

The effect of Antuitrin-S is similar to that of whole sheep's pituitary, which was found by Buyse and Burns[1] to be effective in ovulating the Mexican axolotl. They did not treat the males.

6658

Hypertrophy of the Adrenals in Scurvy.

ARMAND J. QUICK.

From the Department of Surgery, Fifth Avenue Hospital.

The author[1] observed that a marked hypertrophy of the adrenals occurred in guinea pigs that died of scurvy. The same observation had been previously made by Bessesen[2] and others. Svirbely and Szent-Gyorgyi[3] and Waugh and King[4] have demonstrated that the hexuronic acid isolated from the adrenal cortex is vitamin C. It seemed desirable to investigate further the effect of a vitamin C free diet on the adrenal. The results are recorded in Table I.

TABLE I.

Guinea pig No.	Initial wt. gm.	Final wt. gm.	Survival period days	Wt. of Adrenals Right mg.	Left mg.
1	325	235	35	230	250
2	335	240	24	170	200
3	315	240	21	170	240
4	325	250	30	130	140
5	295	190	29	135	160
6 Control)	280	400	33	95	100
7 ”	305	365	26	90	115

The control animals received in addition to the Sherman, La Mer, and Campbell diet, 5 cc. of orange juice daily.

The adrenal hypertrophy is probably an attempted compensatory though futile response on the part of the organism to vitamin C deficiency. The adrenals of the normal guinea pig when cross sectioned and covered with 0.04% of silver nitrate became deeply stained due to the reducing action of the hexuronic acid as Szent-Gyorgyi[5] first demonstrated. It was found that the adrenals of the scorbutic animals, in marked contrast, caused no reduction and remained unstained. This seems to indicate a complete depletion of the hexuronic acid in the terminal stages of scurvy. The relationship of vitamin C to the cortical hormone is not known, but it is interesting to note that in acute cortical deficiency, a marked capillary permeability is observed,[6] while in scurvy there is presumably also an alteration in the permeability of the capillaries resulting in diffuse hemorrhages.

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[1] Buyse, A., and Burns, R. K., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 80.

[1] Quick, A. J., *J. Biol. Chem.*, 1933, **100**, in press.

[2] Bessesen, D. H., *Am. J. Physiol.*, 1922, **43**, 245.

[3] Svirbely, J. L., and Szent-Gyorgyi, A., *Nature*, 1932, **129**, 576.

[4] Waugh, W. A., and King, C. G., *Science*, 1932, **76**, 630.

6659

Relation of X-rays to Lymphomatosis.*

J. FURTH, R. R. RATHBONE AND H. R. SEIBOLD.

From the Henry Phipps Institute and the Department of Radiology, University of Pennsylvania, and the Department of Pathology, Cornell University Medical College.

Five transmissible strains of lymphomatosis were studied. Two strains can be successfully transmitted to any stocks of mice, 3 to related mice only. Mice of stocks yielding from 50 to 100% success upon inoculation with malignant lymphocytes will be designated as susceptible, mice of a stock to which lymphomatosis could not be transmitted will be designated as resistant.

A single exposure of mice to X-rays will increase the susceptibility of the animals to transmissible lymphomatosis. The smallest quantity of X-rays that made resistant mice susceptible to lymphomatosis was about 30 r-units but this amount was effective in only 2 of 13 irradiated mice. The success of inoculations is greater after exposure to larger quantities of X-rays and is about 100% after exposure to sublethal doses (400 to 600 r). The duration of susceptibility is likewise proportional to the quantity of irradiation.

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[5] Szent-Gyorgyi, A., *Biochem. J.*, 1928, **22**, 1387.

[6] Swingle, W. W., Pfiffner, J. J., Vars, H. M., Bott, P. A., Parkins, W. M., *Science*, 1933, **77**, 64.

* This investigation has been supported by a Fund for the Study of Leucemia and Related Diseases.

About one-third of the mice exposed to sublethal doses of X-rays remained susceptible for 2 or 3 months, but more than one-half of them regained resistance in from 1 to 2 months after irradiation.

A single exposure to sublethal doses of X-rays almost completely destroys the leucocytes of the blood and blood-forming organs but it does not prevent the progressive growth of malignant lymphocytes introduced before such an irradiation into the circulation of healthy mice. The inoculated mice often die of lymphomatosis before there is regeneration of the blood-forming organs that were injured by X-rays. Table I summarizes the results of 3 experiments made with one transmissible strain of lymphomatosis (Rg 10) on the effect of irradiation before and after inoculation.

TABLE I.

Mice	Moderately susceptible stock			Resistant stock		
	No. mice injected	Successful No.	Injections %	No. mice injected	Successful No.	Injections %
Non-irradiated	18	5	27.8	13	0	0
Irradiated						
before inoculation	10	8	80.0	14	12	85.7
after inoculation	16	8	50.0	18	3	16.7

Irradiation after inoculation increased the percentage of successful inoculations of mice of a resistant stock from 0 to 16.7% and of mice of a moderately susceptible stock from 27.8 to 50%. Inoculation was even more successful with mice both of a resistant and of a moderately susceptible stock (80 to 85%) when the animals were irradiated before injection.

The results of these experiments suggest that many resistant animals inactivated the malignant lymphocytes that were introduced into their circulation during the interval that elapsed between inoculation and irradiation (2 to 2½ hours).

Irradiation of mice after inoculation prolongs the duration of illness. The average length of life of 11 mice irradiated after injection was 15.8 days, that of 13 mice irradiated before injection was 11.9 days.

Similar experiments were performed with a strain (A 35) which can be successfully transmitted to almost any non-irradiated mice. In 2 experiments with this strain the average length of life after injection of 10 non-irradiated mice was 9.4 days, of 12 mice irradiated before injection 9.4 days, of 18 mice irradiated from 2½ to 24 hours after injection 13 days. Similar results were obtained in experiments made with 2 other transmissible strains.

Prolongation of the duration of illness is probably due to a direct effect of the X-rays on the malignant lymphocytes and may be explained by assuming that some malignant lymphocytes are destroyed by irradiation, the length of life varying inversely with the dose. In most of our experiments irradiation of mice after inoculation of stock susceptible to lymphomatosis did not decrease the number of successful inoculations. In a small number of experiments a decrease of successful inoculations occurred and since the number of successful inoculations is proportional to the quantity of injected cells, it may be assumed that X-rays have destroyed some malignant lymphocytes.

The results of these experiments indicate that X-rays lower the resistance of mice to malignant lymphocytes and suggest that X-rays diminish the number of inoculated leucemic cells but seldom if ever destroy al[1] the cells that have been introduced.

6660

A Secondary Bio-Electric Effect of Potassium.

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The usually large bio-electric effect of potassium salts is often ascribed to the high mobility of K^+ ion in the protoplasmic surface It sometimes appears to be more complex, however, involving the mobility effect as an initial stage, with a secondary effect due to the deeper penetration of potassium. The two steps are often fused into an almost continuous curve in *Nitella*, but are more clearly distinguished in the closely related *Chara coronata*. In the cells of this plant the initial effects of KCl and NaCl are almost identical. The P. D. across[1] the protoplasm (100 to 150 mv. outside positive) is quickly reduced 40 or 50 mv. when tap water is replaced by 0.01 M NaCl or KCl, and then remains at this level for some time. During this time the polarization response remains about as quick and as large as in tap water, so that the effective resistance is high (*e. g.*, 250,000 ohms) to small direct currents passing in either direction across the protoplasm. If an outward current be increased beyond the threshold of stimulation, or if an action current occurs spontaneously, the P. D. quickly falls to about zero. The polarization response also disappears, so that the resistance is entirely ohmic and very low (20,000 ohms).

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[1] For technique see earlier papers in the *J. Gen. Physiol.*

Up to this point the effects of NaCl and KCl are almost identical. In the presence of NaCl recovery begins within a second or 2 and is completed in 5 to 30 seconds, with restoration of the normal P. D. and polarizability. But in the presence of KCl no such recovery occurs: the P. D. remains at about zero and polarization is absent even for rather large currents.

The secondary potassium effect is therefore the inhibition of recovery after an action current. There is increasing evidence that in another plant, *Halicystis,* electrical effects analogous to stimulation occur when the inner surface of the protoplasm is exposed to solutions made alkaline beyond a sharply critical point, recovery occurring when acidity is restored. Outward currents apparently cause this increased alkalinity, inward ones increased acidity. The penetration of ammonia and other bases can cause effects analogous to stimulation and can inhibit recovery.

It is therefore suggested that in *Chara* and *Nitella* the secondary effect of potassium is also to prevent the increase of acidity during recovery from an action current. Its specific prevention of the production of acid (as in iodo-acetic poisoning of muscle) seems unlikely, since potassium is already present in large concentration in the sap. It may simply act by its entrance as a base, neutralizing acids of the cell. This would be expected whether it entered in molecular form (as KOH), or by exchange of K^+ ions for H^+ ions. But the latter seems unlikely since in *Chara,* Na^+ has about the same mobility (initial effect) as K^+ and should therefore exchange as rapidly with H^+. Yet NaCl does not inhibit recovery.

If potassium penetrated very rapidly as base it should itself produce an action current (without recovery); this seems to be usually the case in *Nitella.* In *Chara* (1) it does not enter so fast; (2) the protoplasm may be buffered with more acid; or (3) the stimulation threshold may be at a more alkaline point. The secondary potassium effect is therefore delayed. It may be assisted by an outward current which increases alkalinity; and conversely the potassium inhibition may be counteracted by extremely large currents (*e. g.,* under tension of 0.5 to 1.0 volt) passing inward. The P. D. then rises to high positive values as long as such currents flow, which may be attributed to an increased acidity.

Recovery from the secondary potassium effect occurs slowly when NaCl or tap water is restored. This may be due to the exit of potassium, to the production of more acid by the cell, or to both.

6661

Temperature of Circulating Blood and Other Tissues with Associated Observations of Capillary Activity. *

IRVING S. WRIGHT AND HARRY J. JOHNSON.
(Introduced by H. O. Mosenthal.)

From the Department of Medicine, New York Post-Graduate Medical School and Hospital.

Studies of tissue temperature have been made by means of fairly accurate instruments since the work of Becquerel and Breschet.[1] Interest in recording the temperature of the circulating blood in humans has been secondary to the development of scientifically accurate apparatus which has been devised on thermo-electric principles. Clark[2] developed a galvanometer deflection method of measurement. Harris and Marvin,[3] and Bradburn and Blalock[4] studied changes in the temperature of the venous blood of the hand after immersion in water at various temperatures—by means of direct measurement. Foged[5, 6] reported observations on 116 patients concluding that the temperature in the ulnar vein averaged 2.6 lower than the rectal temperature. He noted, in the presence of fever, an increase in this difference and also that the temperature of the surrounding medium affected the temperature of the venous blood. Other of Foged's observations will be discussed in reference to our findings.

Our observations made on humans were recorded by means of one recording and one non-recording potentiometer (Leeds and Northrup). The readings were taken by means of a thermocouple using the metals copper and constantan at the junction. This thermocouple was contained in the tip of a No. 20 hypodermic needle and the same thermocouple was used for all readings taken in one individual. Mouth temperature was used instead of rectal—making sure that the thermocouple junction rested firmly against the under surface of the tongue. This was done because (1) the relationship between mouth and rectal temperature has been fairly well estab-

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* This work was aided by grants from the Josiah Macy, Jr., Foundation, the Oliver Rea Fund, and the Harriet Weil Fund.

[1] Becquerel and Breschet, *Comptes Rend. de l'Acad. des Sciences*, 1835, **1**, 28.

[2] Clark, H., *J. Exp. Med.*, 1922, **35**, 385.

[3] Harris, K. E., and Marvin, H. M., *Heart*, 1927, **14**, 49.

[4] Bradburn, H. B., and Blalock, A., *Am. J. Physiol.*, 1929, **91**, 115.

[5] Foged, J., *Skandinav. Arch. f. Physiol.*, 1930, **59**, 109.

[6] Foged, J., *Hospitalstidende*, 1930, **73**, 1079.

lished; (2) the use of the needle made rectal readings more difficult and possibly uncomfortable; (3) it was easier to be certain that the tip of the needle rested against known tissue in the mouth; (4) changing the thermocouple or from thermocouple to mercury thermometer would introduce another possible source of error.

The room temperature was maintained between 20.5°C. and 24.5°C.

Intravenous temperatures were taken from the median cubital vein, jugular vein, and branches of the saphenous vein; intramuscular reading from the gluteal or deltoid muscles which were found to be practically identical.

Series I included 30 afebrile patients, studying the relationship between the mouth, intravenous and intramuscular temperatures. In all cases, and in all veins above mentioned the intravenous temperature was lower than the mouth temperature. In the 13 cases tested, the mouth temperature was lower than the intramuscular temperature (with one exception—case 4, which may have been due to error in position of needle). The lowest intravenous temperature reading was 84°F. (28.8°C.) which was doubly checked. Mouth temperature was also low in this patient, 97°F. (36.1°C.) but the difference of 13°F. was the greatest observed. The least difference between mouth and intravenous was noted in case 21—1.2°F. The greatest difference noted between mouth and intramuscular was found in case 7—2.2°F.

Series II represented a study of the relationship between mouth, intravenous and intramuscular temperatures in febrile cases; 13 cases were studied in 3 of which only the mouth and intravenous temperatures were taken. In these cases the fever was due to a disease process, *i. e.,* pneumonia, Hodgkin's disease, rheumatic fever, pulmonary tuberculosis, bacterial endocarditis, etc. All the temperatures were increased, the same general relationship maintaining, namely: Intravenous lowest, then mouth, and intramuscular temperature highest. We can establish no consistent change in relationship. The consistently highest average held by the intramuscular temperature tends to confirm the belief that the muscles are local generators of heat by metabolic activity.

Series III represented a study of the relationship of the subcutaneous temperature to the mouth, intravenous and intramuscular temperatures. Eight patients were used. In our cases the subcutaneous temperature was found to be either higher or lower than the intravenous temperature, but never equal to the mouth or intramuscular temperatures.

CHART I.
Intra-arterial Temperature—Relation to Mouth and Intravenous Temperature.

Patient	Mouth Temp.		Intravenous Temp.		Intra-arterial Temp.	
	F	C	F	C	F	C
1	99.6	37.5	96.0	35.6	97.25	36.4
2	98.4	36.8	92.0	33.3	92.75	33.7
3	101.5	38.6	93.0	33.9	99.0	37.2
4	105.5	40.8	98.0	36.6	103.2	39.6

Chart I gives the figures of 4 intra-arterial temperatures taken in the radial artery and compared with the mouth and intravenous temperatures of the same patients. Foged reports 2 intra-arterial temperatures, one of which equaled the rectal temperature, and one of which was 0.3°C. lower than rectal temperature, and concludes that the arterial temperature is apparently the same as the rectal temperature. We have found the arterial temperature (in febrile and afebrile cases) to be between the intravenous and mouth temperature but not equal to the mouth (and hence rectal) temperatures.

Two cases were studied in which the temperature was taken in one area in the gluteal region beginning with the skin and progressing inward to the muscle, and comparing it with the intravenous and mouth temperatures. The skin was found to be higher than the subcutaneous temperature. The intramuscular was the highest in both cases, and the intravenous the lowest in one case and equal to the skin in the other.

CHART IIa
Effect of Fever Produced by Diathermia on the Mouth, Intravenous and Intramuscular Temperatures. (Hyperdiathermia Machine—General Electric)

Patient	Mouth Temp.		Intravenous Temp.		Intramuscular Temp.	
	F	C	F	C	F	C
1						
Normal	98.6	37.0	95.4	35.2	99.2	37.37
Peak of Fever	105.0	40.5	103.5	39.65	106.0	41.15
2						
Normal	98.2	36.9	94.4	34.6	100.0	37.7
Peak of Fever	102.8	39.2	99.5	37.25	104.4	41.0

CHART IIb. (Chart IIa continued)
Effect of Fever Produced by a 36 meter Wave Length Condenser Machine.
(Hyperthermy spark gap—Lepel)

Patient	Mouth Temp.		Intravenous Temp.		Intramuscular Temp.	
	F	C	F	C	F	C
1						
Normal	98.0	36.8	96.0	34.6	98.0	36.8
Peak of Fever	104.4	40.2	102.5	39.2	104.5	40.3
2						
Normal	98.5	36.99	95.0	35.0	99.0	37.25
Peak of Fever	103.0	39.4	101.5	38.47	103.8	39.85

Chart IIa presents the mouth, intravenous and intramuscular temperatures of 2 patients in whom fevers were produced by means of the Hyperdiathermia Machine (General Electric). The temperature rises in each area reached the peak in 30 minutes after taking the normals.

Chart IIb presents the same statistics on 2 cases in which the fever was induced by means of a 36 meter wave length condenser machine (Hyperthermy spark gap—Lepel). The results are very similar in all readings.

CHART III.

Mouth and Intravenous Temperature Changes in Relationship to Changes in Capillary Activity during Fever Produced by *B. typhosus* Vaccine (30 million) Intravenously.

Time	Mouth Temp.		Intravenous Temp.		Capillary Activity (Nailfold)
	F	C	F	C	
12 noon	98.0	36.6	92.75	33.7	
12:10	98.4	36.8	91.8	32.8	Few capillaries seen per field. Straight or slightly tortuous loops. Flow moderately rapid. Moderate dilatation. Occasional stoppage.
1:10	98.6	36.9	92.0	33.3	
2:30	99.9	37.7	93.0	33.9	
3:00	100.4	37.9	92.5	33.6	
3:30	100.4	37.9	92.5	33.6	
4:00	100.2	37.8	93.5	34.2	
4:20	100.0	37.7	95.0	34.9	
5:00	100.0	37.7	96.5	35.8	
					Estimated to be 4 times as many capillaries active per field. Flow rapid. No stoppage. Greater dilatation. Arterioles and venules seen with anastomoses active.

Chart III represents the mouth and intravenous temperature as taken on a recording potentiometer during the induction of fever by means of the intravenous injection of 30 million *B. typhosus* in vaccine. Both the mouth and intravenous temperatures rose but the intravenous temperature was observed to lag, showing no definite increase until 4 p. m., 3 hours and 50 minutes after the first increase noted in the mouth temperature. The thermocouple was left in the vein throughout this experiment so that the first changes could be noted. After 4 p. m. the mouth temperature started to decline but the intravenous temperature still continued to mount until the experiment was discontinued at 5 p. m. (4 hr. and 50 min.).

This intravenous lag was not noted in the preceding series, Chart II, and where fever was induced by other methods. We have not made continuous observations on rising temperature due to disease processes.

The intravenous temperature is subject to fluctuations of 2-4°F.

within a period of 2 minutes. These were in the form of a sudden drop and restitution to former level. When the intravenous temperature began to rise these fluctuations became more frequent, occurring as often as every 10 minutes.

This chart also illustrates diagramatically the great increase in capillary activity with the production of fever. The lower blood temperature would incline us to believe that the purpose of this was not a direct increase of local heat but rather a means of supplying the cells, especially of the muscles, with an increased food supply for metabolic activity and associated heat generation.

6662

A Comparison of the Value of Ferrous Iodide Administered Directly and Indirectly.*

HAZEL C. CAMERON. (Introduced by A. J. Carlson.)

From the Agricultural Experiment Station, West Virginia University.

Ferrous iodide has been administered orally as a tonic for many years in the practice of medicine without any evidence of toxicity when therapeutic doses are not exceeded. Recently, however, "toxicity" on direct oral administration has been ascribed to it in vitamin A deficient rats[1] in explanation of the negative results obtained by Mason,[2] Cameron,[3] and Mendel[4] when ferrous iodide was used as a substitute for vitamin A.

In all of these experiments, dilute ferrous iodide was given *by pipette or syringe* to rats depleted of vitamin A, a method which prompted the criticism of toxicity. In the following experiments, comparison is made of the effects of ferrous iodide when given by this method and when given as a part of the diet, thus avoiding any possible toxic effect of direct oral administration.

In these experiments dilute syrup of ferrous iodide alone or combined with linoleic acid has been given daily, mixed with a small amount of dry diet in a small glass dish left in the cage without other food until its contents was consumed. The results are compared with the earlier experiments in which direct oral administration was used. Standard vitamin A procedure was followed,[3] vitamin D being supplied throughout by the addition of 0.05 mg. of irradiated ergosterol per 1000 gm. of diet. At the first appearance of xerophthalmia, usually between the 8th and 9th weeks, administration of ferrous iodide or other supplement was begun and continued to the death of the animal when autopsy was done. Observations were made 3 times a week to detect improvement in the eyes. The following supplements were used: syrup of ferrous iodide diluted 1-350, 5 drops daily; linoleic acid, 4 to 6 drops; linoleic acid and dilute ferrous iodide mixed in equal amounts, 8 drops daily. The drops were measured from the same pipette in all cases.

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* Published with the approval of the Director, West Virginia Agricultural Experiment Station, as Scientific Paper No. 121.

[1] Chidester, F. E., *Collecting Net.*, 1932, **7**, 229.

[2] Mason, Karl E., *Anat. Rec.*, 1931, **51**, Supplement 1, Abst. 91.

[3] Cameron, H. C., *Science*, 1932, **76**, 18.

[4] Reed, Lucille L., Mendel, Lafayette B., and Vickery, H. B., *Science*, 1932, **76**, 300.

Figure 1 shows the effect of the various supplements on growth. It is evident that death as represented by the end of the curve, comes at almost exactly the same time whether ferrous iodide is given by

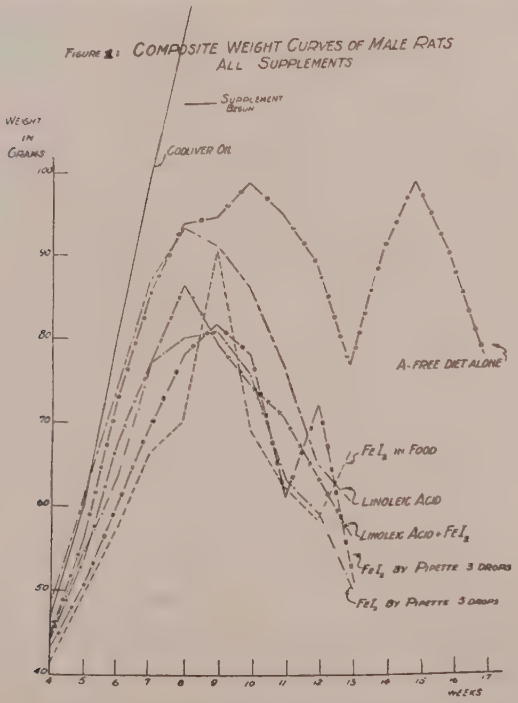
FIGURE 1: COMPOSITE WEIGHT CURVES OF MALE RATS
ALL SUPPLEMENTS

pipette or mixed in the diet, alone or with linoleic acid. The break in the curve with an apparent gain in weight just before death, does not occur in the growth curves for individual rats, and seems to be caused in the composite curves by the diminishing numbers of rats toward the end of the experiment. The peak of growth occurs in most cases before any supplement is given and declines almost immediately thereafter to the death of the animal. The contour of the individual curves shows a striking similarity in both male and female rats. In all cases, the untreated vitamin A-free rats live longer than their supplemented litter mates. There is thus no evidence of any greater "toxicity" for ferrous iodide given by pipette than in the diet; and no evidence of growth promoting effect from any of the supplements. This is in striking contrast to codliver oil which maintains continuous growth. There was no evidence of improvement in xerophthalmia or of middle ear and tongue infections which occurred in all of the vitamin-free rats, treated or untreated.

The figures given in Table I, for the mean age at death of the animals in each group, bear out the observations made from the growth curves. In general, the females in all groups tended to live a few days longer than the males and to weigh a few grams less at death; but since the differences were small, figures for male and female rats have been combined. It is evident from these figures that neither ferrous iodide alone or combined with linoleic acid prolonged the life of vitamin A-free rats, since the unsupplemented rats of Group I—those on vitamin A-free diet alone—lived longer than any other lot. None of the differences are large in the life of a rat, but *all are in favor of the untreated rats.*

At first glance, it may be thought that ferrous iodide by mouth has hastened the death of the animals, since those receiving 3 drops by pipette died earlier than any other group. However, comparison of the figure for 23 rats given 5 *drops by pipette* with that for

TABLE I.
Comparison of Age at Death of Treated and Untreated Rats (Male plus Female)

Group	Treatment	No. Rats	Age at Death		Deviation from Group I		
			Mean	P.E.	Mean	P.E.	$\frac{D}{P.E.}$
I	Unsupplemented Rats	32	92.06	1.75	—	—	—
II	FeI_2 given by pipette (3 gtts.)	22	76.73	1.01	15.33	2.02	7.59(–)
III	FeI_2 given by pipette (5 gtts.)	23	85.57	1.98	6.49	2.64	2.45
IV	FeI_2 mixed in diet (5 gtts.)	37	87.84	1.13	4.22	2.08	2.03
V	FeI_2 plus Linoleic Acid in food	21	90.29	0.97	1.77	2.00	0.89
VI	Linoleic Acid alone in food	24	88.00	1.17	4.06	2.10	1.93

37 rats given 5 *drops in the diet* shows a difference of only 2 days, which is certainly not indicative of toxicity. The discrepancy in the 3-drop group must therefore be sought elsewhere.

Review of all the data from the statistical standpoint verifies these conclusions. Assuming that the individual figures are sufficiently uniform to make a smooth frequency curve, a mean difference between 2 groups that is 4 times its probable error offers odds of *142* to 1 that the difference is significant and could be obtained again under like conditions. In column 4, Table I, is given the difference, with its probable error, between the mean age of the untreated rats of Group I and that of each of the other groups. The last column gives the ratio of the mean difference to its probable error. It will be seen that only one difference is more than 4 times its probable error, and the difference in this case *is in favor of the untreated animals* (marked –). This is true in every instance in the figures for male and female rats considered separately (not shown here), and for weight at death as well as for age at death. Comparison of the age at death of all rats given ferrous iodide by pipette (5 drops) with those given ferrous iodide in the food (5 drops) reveals a mean difference of 2.27± P. E. of 2.28. There is thus no significant difference between the 2 methods of administering the ferrous iodide. With respect to weight at death, the mean difference between the 2 groups is 5.59± P. E. of 1.46, or a ratio of 3.82 to 1 *in favor of the animals fed by pipette.*

Conclusion. There is thus found to be no significant difference in response of animals to ferrous iodide fed by pipette or mixed in the diet, and no evidence of a "toxicity" factor from the former. Furthermore, no vitamin-A substituting effect is obtained with ferrous iodide alone or combined with linoleic acid given either by pipette or mixed in the food.

6663

Late Effects of Pinealectomy in Rats.

ARTHUR D. RENTON AND HAROLD W. RUSBRIDGE.
(Introduced by J. C. Donaldson.)

From the Department of Anatomy, School of Medicine, University of Pittsburgh.

Izawa[1] reviewed the work on pinealectomy and added the results of his own operation on rats. He pinealectomized his animals at 20 days of age and killed them at 85 days. His results showed accelerated growth of the gonads in the male and female, a possible retardation in the growth of the eyeballs in both, and an increase in weight of the hypophysis in the male. No effects on the other endocrine glands were noted. It seemed desirable, in the light of these results, to see whether these effects were permanent in the adult animal or transitory conditions present only during the time of active growth.

Sixteen albino rats, 11 females and 5 males, were pinealectomized at about 26 days of age. A set of litter-mate control rats was subjected to a similar operation but without actual pinealectomy. Non-operated litter-mate controls were also kept. The rats were autopsied about 340 days after operation. Absence of the pineal was confirmed by histological examination of the brain.

The pituitary, thyroid, adrenals, testes, ovaries, epididymides, and eyeballs were examined grossly and weighed. Careful comparison was made with the organs from the control and operated control animals and the results compared with the weights given for the Wistar Standard rats (Donaldson[2]). No consistent or significant changes were observed. In addition, the adrenals were measured and the relative proportions of cortical and medullary substance determined. Again no change from the normal could be detected.

There is no evidence, therefore, of any major persistent effect on any of the organs examined following removal of the pineal. Although our series was limited, we believe that it was large enough to show any positive results which might have been carried over from Izawa's growing rats to our adult series.

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[1] Izawa, Y., *Am. J. Physiol.*, 1926, **77**, 126.

[2] Donaldson, H. H., The Rat, 1924.

6664

Vitamin A Deficiency in the Dog.

HAROLD S. OLCOTT.* (Introduced by Lafayette B. Mendel.)

From the Department of Physiological Chemistry, Yale University.

Steenbock, Nelson, and Hart[1] produced xerophthalmia in dogs on a diet free of fat-soluble vitamins. Suzman, Miller, and Ungley,[2] and Ralli and Waterhouse[3] were unable to do so but noticed anorexia and dermatitis after dogs had been fed diets lacking vitamin A. In view of these contradictory results, it seems desirable to record another unsuccessful attempt to produce xerophthalmia in the dog. Three female dogs of the same litter, 3 months old, were placed upon a diet containing casein (alcohol-extracted), 25%, dextrin, 10%, sucrose, 30%, lard, 25%, vitavose,† 6%, bone ash, 3%, and salt mixture,‡ 1.5%; each dog was given 2 drops of viosterol daily in addition to the ration of 25 gm. per kilo. Diets similar to this, when supplemented with cod liver oil, have been used with complete success in numerous other experiments with dogs.[4] The animals gained weight for approximately 2 months. About that time, appetite became sporadic and the animals rarely ate their entire daily ration. Two of the dogs died within a day of each other, having been on the diet 77 days. The only observable symptom preceding death, and that for only one day, was extreme weakness and lassitude. The third dog died 25 days later under identical conditions. There were no discoverable abnormalities in any of the 3 autopsies.

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* National Research Council Fellow in Medicine, 1931-1932.

[1] Steenbock, H., Nelson, E. M., and Hart, E. B., *Am. J. Physiol.*, 1921, **58**, 14.

[2] Suzman, M. M., Muller, G. L., and Ungley, C. C., *Am. J. Physiol.*, 1932, **101**, 529.

[3] Ralli, E. P., and Waterhouse, A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, in press.

† A wheat germ preparation of E. R. Squibbs and Sons, New York.

‡ NaCl, 25 parts, KCl, 25, Ca lactate, 22.5 Mg. citrate, 22, ferric citrate, 5, KI, 0.5.

[4] Cowgill, G. R., *J. Biol. Chem.*, 1923, **56**, 725; *Am. J. Physiol.*, 1923, **66**, 164; Cowgill, G. R., Rosenberg, H. A., and Rogoff, J., *Am. J. Physiol.*, 1931, **96**, 371.

6665

Rapid Production of Highly Potent Scarlatinal Toxin.

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E. H. TAYIAN.

From the Department of Immunology, Evans Memorial, Massachusetts Memorial Hospitals, Boston.

In producing mass cultures of streptococci and pneumococci, Mueller and Klise[1] found it distinctly advantageous to maintain approximate neutrality of cultures growing in 2% glucose broth; this was accomplished by frequent additions of sterile NaOH. The yield of cocci and/or of soluble specific substance was considerably enlarged. We have applied their method in a study of the production of other bacterial products—the erythrogenic toxins and the lysin of the N.Y.5 strain of hemolytic streptococcus.

Comparisons of the activities of this strain in 1 L. quantities of tryptic digest broth, pH 7.9, 3.82 mg. N_2/ml, were made under 3 conditions: I, 2% glucose and phenol red were added to basic medium and, after growth began, the color was kept faintly pink by repeated small additions of 10% NaOH—84 ml were used in 24 hours; II, basic medium containing indicator and 2% glucose; III, basic medium and indicator. Each 2 L. flask was seeded with 5 ml of a 6 hour broth culture; at successive 4 hour intervals, samples were withdrawn for tests. Observations are recorded in the figure and table.

Hemolytic activity of centrifugates was at once measured at 37°C. in the presence of 0.5 ml of 5% rabbit cells; total volume, 1 ml; incubation, 1 hr.

TABLE I.
Observations on pH, N_2, and glucose contents, viability, and suspension stability of cultures.

		I	II	III
pH, 24 hr.		7.5	5.47	5.92
Glucose	12 hr., mg/ml	2.2	15.3	
	24 hr., mg/ml	1.9	11.7	
N_2	24 hr. mg/ml			
	Whole culture	3.77	3.71	3.59
	Filtrate	3.23	3.23	3.16
Viable cocci, 24 hr.				
	0.00001 ml	Overgrown	0	1
	0.0001 ml		0	20
	0.001 ml.		6	168
Suspension		Smooth	Flocculent	Flocculent

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[1] Mueller, J. H., and Klise, K. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 454.

The bacterial bulks were measured by uniform centrifugation and are charted as volume percent. The cocci in lot 1 were notably small and usually gram-negative; used as a bacterial vaccine this

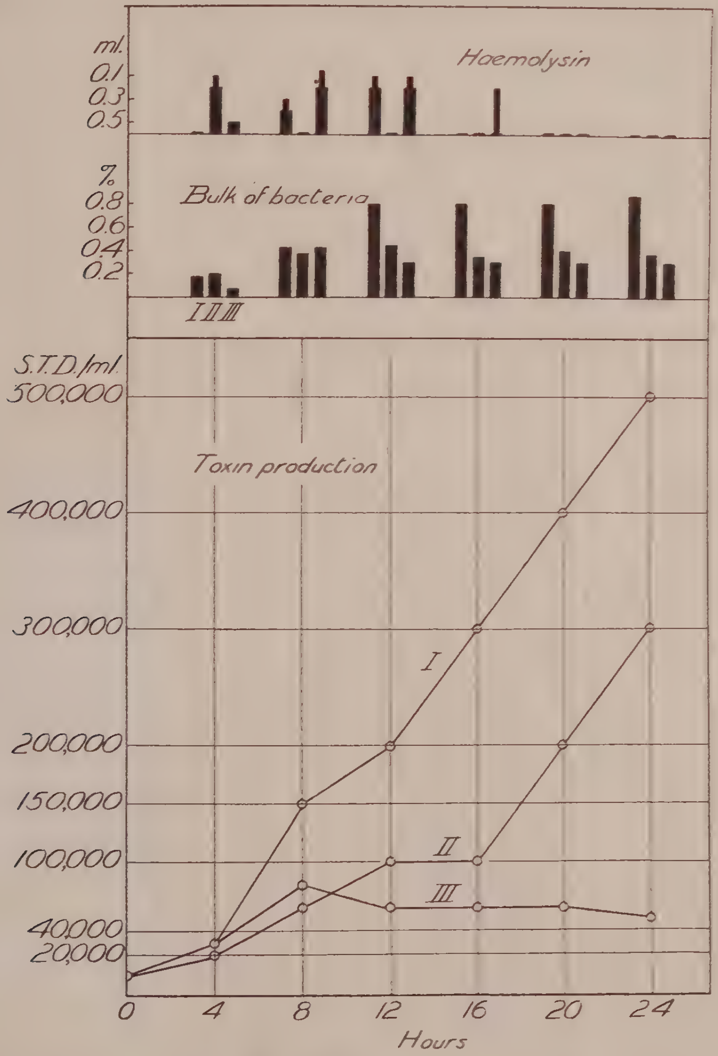

FIG. 1.
Observations on growth, toxin and lysin formation.
(The symbols II and III, *relating to toxin production*, should be interchanged.)

suspension has evoked larger local reactions than ordinary suspensions of apparently the same density.

Toxins were titrated on subjects who responded with bright, 20 mm. reactions. Estimates of the skin-test dose (STD) are considered accurate only within 50%. The potency of toxin "B",[2] also produced by this strain, was measured and found to parallel that of the Dick toxin ("A") except that in lot II after 8 hours it rapidly declined and at 20 hours there were less than 100 STD/ml of "B" toxin remaining. This behavior affords an additional criterion of differentiation between "A" and "B" toxins. The stability of toxins prepared as in lot I does not appear to differ markedly from that of toxins prepared in the ordinary way; after 3 months there was about a 50% loss in potency.

Among other things the observations indicate a connection between rapidity of growth and toxigenesis. They do not suggest identity of hemotoxin with erythrogenic toxin.

6666

Attempts to Produce Hyperplasia of Thyroid Gland by Infection and by Injury to the Adrenal Gland.

HOWARD R. MAHORNER. (Introduced by Alton Ochsner.)

From the Department of Surgery, Tulane University School of Medicine.

The etiology of exophthalmic goiter is still unknown. However, we do know one change that occurs regularly in typical exophthalmic goiter—hyperplasia of the thyroid gland. Various theories have been proposed as to the basic factor underlying this hyperplasia. In these experiments I have been interested in two: (1) Infection; (2) Disordered action of the adrenals.

Halsted[1] found hyperplasia of the thyroid gland after producing in dogs a low grade peritonitis by the intraperitoneal injection of staphylococci. Later he thought he could attribute certain hyperplastic changes in the thyroid to infection of the wound. Cole and Womack[2] described hyperplasia of the thyroid occurring after the implantation of infected foreign bodies into the neck muscles of dogs.

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[2] Hooker, S. B., and Follensby, E. M., *J. Immunol.*, 1928, **15**, 601.

[1] Halsted, *Am. J. Med. Sc.*, 1914, **147**, 56.

[2] Cole, and Womack, *J. Am. Med. Assn.*, 1929, **92**, 453.

Marine and Baumann[3] found an increase in the basal metabolic rate in some instances after removal of or freezing the adrenal glands of rabbits.

Dogs were used in my experiments. At a preliminary operation I exposed the thyroid lobes, measured them, and took a piece of tissue, 2x2 mm., from the anterior border of the right lobe. Controls showed that no hyperplasia resulted from the removal of this much gland.

In the experiments on the effect of infection on the gland, I implanted foreign bodies contaminated with a culture of staphylococci or with a mixed culture of organism obtained from a fecal fistula into the neck muscles of 8 dogs. These dogs were sacrificed subsequently at periods varying from 4 to 64 days. All except one showed inflammatory reaction around the foreign body. On comparing the necropsy specimen of the thyroid grossly and microscopically with the thyroid at the time of the first operation, no definite evidence of hyperplasia could be observed. I previously reported[4] hyperplasia in 50% of the instances after infection. I have reviewed these slides and now do not think there is any definite evidence of hyperplasia.

In other series of dogs, after removing in a preliminary operation a small 2x2 mm. piece of tissue from the lobe of the thyroid, the adrenals were crushed, destroying from one to 1½ of the 2 adrenals. These dogs looked weak and apathetic and just the opposite to the nervous stimulated dogs who have received thyroid extract over a period of time. Observations made on 11 dogs in comparing the sections from the preliminary operation with sections removed at periods varying between 4 and 127 days after injury to the adrenals showed no evidence whatsoever of any hyperplasia, except in one instance where there was only a suggestion, not at all definite, of some slight hyperplastic change. In the early stages the adrenals were partially destroyed, but regenerated after the longer periods.

Similar experiments made upon 7 dogs from 4 to 85 days after freezing the adrenals thoroughly with ethyl chloride showed no evidence of hyperplasia in the thyroid. In the earlier periods there was evidence of hemorrhage and necrosis in the adrenals; later repair was complete.

These results differ from the observations of other authors on the effect of infection on the thyroid and would not substantiate the view that increased basal metabolic rate after injury to the adrenals was the result of hyperactivity of the thyroid gland. I am not aware that any former observers have removed a piece of the thyroid at a preliminary operation in their animals to compare subsequent sections with. It is easily conceivable that the appearances which they observed were not changes from the original state of the thyroid but were present before the experiment began. The results of my experiments seem to show that definite evidence of hyperplasia in the thyroid does not follow the introduction of infection in animals or injury of the adrenal glands by freezing or crushing.

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[3] Marine and Baumann, *Am. J. Physiol.*, 1921, **57**, 135.

[4] Mahorner, H. R., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1076.

6667

Ulceration in Digestive Tract Following Experimental Lesions in Brain-Stem.*

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From the Department of Physiology and Pharmacology, University of Alabama School of Medicine.

The relation of gastro-intestinal ulceration to lesions of the central nervous system has been reviewed by Cushing[1] and Light.[2]

In our series of 50 cats and 40 dogs having experimental lesions in the upper brain-stem, acute gastro-intestinal changes have occurred in a relatively small number. The other animals have exhibited normal guts or slight changes that are difficult to evaluate. Lesions have varied considerably in extent and location. Apart from 3 chronic mid-brain cats in which erosions were found in the stomach, the changes have followed lesions accompanied by hemorrhage into the ventricles or transverse lesions at the level of the chiasm unassociated with intraventricular hemorrhage. This report is confined to a description of maximum reactions noted.

Hyperemia and Hemorrhage. Marked hyperemia of the stomach has been encountered in 12 dogs at death—7 to 24 hours after operation—confined in most instances to the body (Fig. 1). Associated

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* This investigation is being aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Cushing, H., *Surg. Gynec. and Obstet.*, 1932, **55**, 1.

[2] Light, *J. Pharm. and Exp. Ther.*, 1932, **45**, 227.

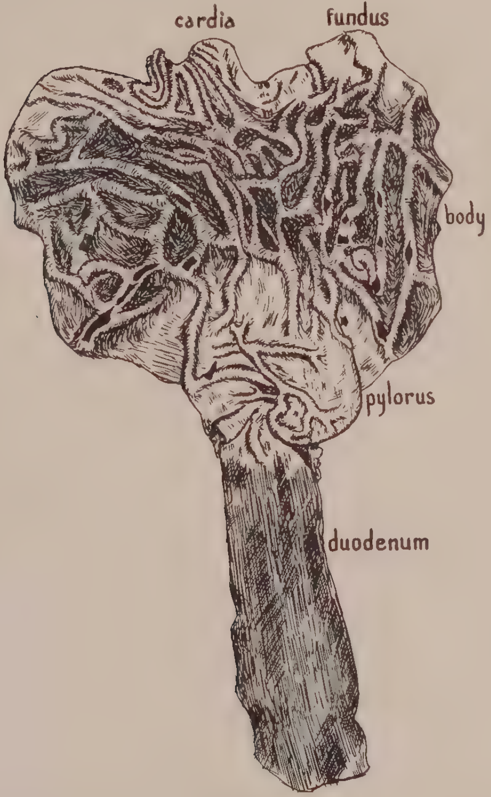

FIG. 1.

with the hyperemia were multiple hemorrhagic spots in the mucosa, marked by attached clots. The hemorrhages occurred most frequently on the crests of the mucosal folds. Blood in the lumen varied from a slight staining of the mucus to large free clots.

Associated with this reaction in the stomach was a remarkable generalized hyperemia of the mucosa of the duodenum. The hyperemia was progressively less marked going distally, until at the upper end of the jejunum it again became more pronounced, fading as the distal ileum was approached. The intestinal content varied from bloody mucus to blood only. (From the small bowel of Dog 27, 50 cc. of practically pure blood was collected at autopsy; blood was the chief constituent of antimortem feces.) In the large bowel hyperemia was confined to crests of longitudinal folds and in cases amounted to frank hemorrhage.

Erosions. Multiple erosions in the stomach were encountered in 8 dogs at death—24 to 60 hours after operation—and in 3 cats at death—1, 2, and 7 weeks after operation. They occurred most frequently on the anterior and posterior surfaces of the body, just proximal to the pylorus, being more numerous on the crests of the folds (Fig. 1). Infrequently erosions occurred in the pylorus and proximal duodenum. Only in 2 instances were the erosions associated with hyperemia and hemorrhage.

Both hemorrhages and erosions have occurred in animals in which there was no vomiting and no hyperthermia.

Enhanced appetite has occurred in animals having relatively small lesions in the hypothalamus. A half-grown kitten consumed 600-700 cc. milk in an 8-hour period, whereas the *maximum* intake of unoperated kittens has been 300-400 cc. The increased intake is not due to thirst since the water intake was only 10 to 50 cc. per day when food was withheld. A few of these animals exhibited spontaneous vomiting which was relieved by food. In 2 cases vomiting was precipitated on occasions by withholding food and then relieved by feeding. (These animals had normal blood sugars; however, in several of the dogs there was a progressive fall in blood sugar to below normal.)

Impaired digestion was manifested in several cats with the hypothalamus severed from the rest of the brain-stem and in one low mid-brain preparation by the presence of undigested meat (no evidence of beginning digestion) in the stomach at autopsy 24 to 72 hours after feeding, and by the appearance of undigested meat in the feces 36 to 72 hours after feeding. No gross pathological changes were observed in these cases.

Cardio-vascular disturbances encountered are: Irregular pulse with forceful beats; immediate ventricular fibrillation when needle was introduced for blood (3 instances); and the finding of large fibrinous clots in the chambers of the heart—right and left—and large vessels. (One of the most marked was a dog with no brain lesion in which blood was injected into the ventricles through the corpus callosum.) In addition fibrinous non-occluding plugs were found in the smaller vessels of the liver, lungs, and stomach.

Our data at present do not allow conclusions as to localization of mechanisms concerned or the manner in which they act; however, we believe that hemorrhage into the ventricles as well as lesions in the anterior hypothalamus unassociated with intraventricular clots are significant, particularly in the light of the work of Beattie,[3, 4] Cushing,[5] Light,[2] and Ferguson.[6]

6669

Increased Potency of Liver Extract by Incubation with Human Gastric Juice.*

O. M. HELMER, PAUL J. FOUTS AND L. G. ZERFAS.
(Introduced by G. H. A. Clowes.)

From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, and the Department of Medicine, Indiana University School of Medicine.

Following the work of Castle and his associates,[1] Reimann[2] reported a marked increase in the potency of liver by its digestion in normal human gastric juice. Walden and Clowes,[3] independent of Reimann, obtained a very active preparation by the incubation of liver and liver extracts with small amounts of hog gastric tissues. However, Barnett and Thebaut[4] were apparently unable to increase the activity of liver by its digestion with normal human gastric juice.

The purpose of this paper is to report the results obtained by this laboratory when patients with pernicious anemia in relapse were fed daily a subminimal dose of liver extract No. 343 (that derived from 100 gm. of liver) which had previously been incubated at 40°C. for 4 hours with 100 cc. of normal human gastric juice obtained after histamine stimulation. During the control and test periods the diets

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[3] Beattie, *Proc. Royal Soc.*, 1930, B, **106**.

[4] Beattie, *Can. Med. Assn. J.*, 1932, **26**, 278.

[5] Cushing, *Proc. Nat. Acad. Science*, 1931, **17**.

[6] Ferguson, PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 328.

* The authors wish to acknowledge the technical assistance of Miss Betty Goss.

[1] Castle, W. B., *Am. J. Med. Sci.*, 1929, **178**, 748. Castle, W. B., and Townsend, W. C., *Am. J. Med. Sci.*, 1929, **178**, 764. Castle, W. B., Townsend, W. C., and Heath, C. W., *Am. J. Med. Sci.*, 1930, **180**, 305. Castle, W. B., Heath, C. W., and Strauss, M. B., *Am. J. Med. Sci.*, 1931, **182**, 741. Strauss, M. B., and Castle, W. B., *New Eng. J. Med.*, 1932, **207**, 55

[2] Reimann, F., *Med. Klin.*, 1931, **27**, 880.

[3] Walden, G. B., and Clowes, G. H. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 873.

[4] Barnett, C. W., and Thebaut, W. M., *J. Am. Med. Assn.*, 1932, **99**, 556.

of the patients contained no meat or eggs. The noon meal was given at 11:30 A. M. and the evening meal at 6:30 P. M. At 4:30 P. M. the material to be tested (after being brought to pH 5 by the addition of sodium hydroxide) was administered by mouth to the patients.

Case I received daily during the 10 day control period the amount of liver extract No. 343 derived from 100 gm. of whole liver. No response in the blood or improvement in the clinical condition of the patient resulted. This confirmed the findings of Zerfas[5] that this amount of liver extract No. 343 is insufficient to cause a reticulocyte response when fed by mouth to patients with pernicious anemia in relapse. During the following 10 day period the patient received daily the same amount of liver extract incubated with gastric juice as described above. Marked clinical and hematological improvement followed. Chart 1 shows the response of the blood to the medication.

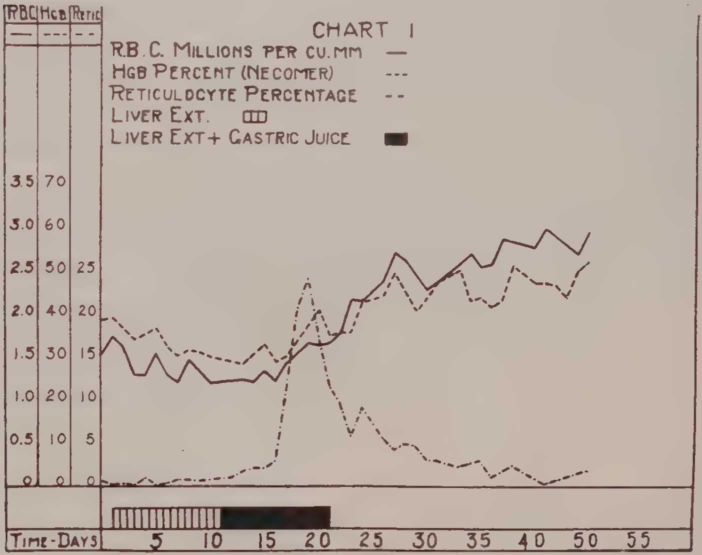

CHART 1.

Response of red blood cells, hemoglobin, and reticulocytes of Case 1 to the amount of liver extract derived from 100 gm. of liver and to the same amount of liver extract that had been incubated at 40° for 4 hours with 100 cc. of normal human gastric juice.

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[5] Zerfas, L. G., *Arch. Int. Med.*, 1931, **47**, 135.

Case II during the 10 day control period received daily by mouth 150 cc. of normal human gastric juice which had been incubated for 4 hours and then adjusted to pH 5. No response in the blood of the patient was noted. This demonstrated that the response obtained in the first patient was not due to the effect of the gastric juice upon the food the patient ate during the experiment and confirmed the work of Castle and his associates that normal human gastric juice is ineffective in the treatment of pernicious anemia when fed by mouth. During the following 10 day period Case II received daily the liver extract-gastric juice preparation described above. Again a very good response in the reticulocytes and red blood cells (Chart 2) followed this medication.

In these experiments a known subminimal dose of liver extract No. 343 after incubation with human gastric juice (itself inactive) produced maximal reticulocyte responses and marked clinical improvement when administered daily by mouth to patients with pernicious anemia in relapse. These results are in harmony with and

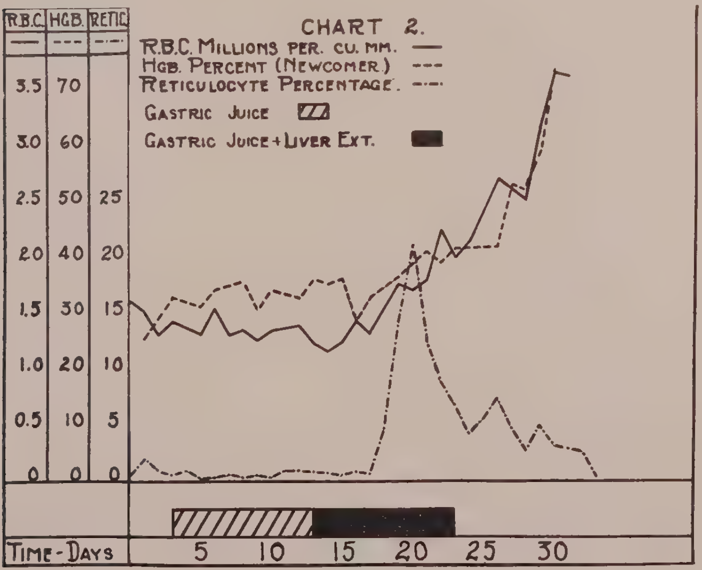

CHART 2.

Response of red blood cells, hemoglobin, and reticulocytes of Case II to 150 cc. of normal human gastric juice incubated at 40° for 4 hours and to liver extract No. 343 derived from 100 gm. of liver that had been incubated at 40° for 4 hours with 100 cc. of normal human gastric juice.

lend confirmation to the observations of Castle and his associates, of Walden and Clowes, and of Reimann. It still remains to be determined whether the enhanced activity which we have observed is due to an increase in the active principle originally present in the liver or to an effect of the type described by Castle and his associates when human gastric juice was allowed to act on beef muscle, beef muscle extracts and yeast.

6670

A Highly Satisfactory Vitamin B Deficient Ration.*

BARNETT SURE.

From the Laboratory of Agricultural Chemistry, University of Arkansas, Fayetteville.

For a number of years we have experienced considerable difficulty in securing uniform and reliable results on so-called vitamin B deficient rations. In all of our vitamin B studies we have negative as well as positive controls. Very frequently the negative control, receiving no vitamin, would continue growing for many weeks, which was quite distressing, particularly in the biological assay of concentrates which necessitated discarding appreciable amount of data, thus involving much waste of time and labor. After numerous attempts to arrive at the source of the troubles we finally discovered 2 main factors responsible for animals growing after the vitamin reserves have been depleted. First, our method of purifying casein by washing for 7 to 10 days in acidulated water is inadequate and second, subjecting dried baker's yeast to such drastic treatment as autoclaving for 8 hours at 20 pounds pressure did not always insure the destruction of all traces of vitamin B. Furthermore, such drastic autoclaving destroyed appreciable amounts of vitamin G.

The purification of commercial casein, purchased from Atterbury Bros., New York, is carried out as follows: 3 kg. of casein are suspended in 30 liters of tap water to which are added 50 cc. glacial acetic acid and 5 cc. chloroform and 5 cc. toluene as preservatives. The water, acetic acid, and preservatives are changed daily for a period of 14 days. During the last 3 days of this period distilled water is used instead of tap water. The casein is then dried at 100°C. and ground. Five kilograms of the dried acid washed casein are subsequently suspended in 25 liters of 25% ethyl alcohol, by volume, and soaked for 24 hours. During this period the casein is stirred in the alcohol for 12 hours with a mechanical stirrer. This operation is conducted at room temperature. The casein is then filtered through a large Buchner funnel, dried at 100°C. and ground.

Our ration deficient in the vitamin B complex (No. 1751) consists of casein (purified according to the method described), 20;

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* Research paper No. 298, Journal Series, University of Arkansas.

agar-agar, 2; McCollum's salts No. 185, 4; filtered butter fat, 10; and dextrin, 64. On this ration, deficient only in the vitamin B complex, we secure depletion in 2 and generally in not more than 3 weeks.

For our uncomplicated vitamin B studies we have introduced autoclaved round steak as a source of vitamin G, because muscle meats are known to be deficient in the antineuritic and abundant in the antipellagric factor. The autoclaving is carried out as follows: Thirty pounds of round steak are cut in small cubes and heated for 6 hours at 20 pounds pressure, dried at 100°C., and ground. The composition of the ration deficient only in vitamin B (No. 2345) is as follows: Casein (purified with acidulated water and 25% alcohol), 10; salts No. 185, 4; autoclaved dried round steak, 15; butter fat, 10; dextrin, 61. On this ration depletion of vitamin B reserves generally take place 2 to 3 weeks after all storage of the vitamin B complex has been depleted on Ration No. 1751. The most satisfying feature about ration 2345 is that all negative controls lose weight on this diet rapidly and invariably die.

We have determined that this ration is abundant in vitamin G for optimum growth with a large number of animals, receiving supplementary additions of small amounts of highly concentrated vitamin B preparations.

6671

Further Studies on Production of Thecal Luteinization by Means of A. P. L.

H. SELYE, J. B. COLLIP AND D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal.

We have reported that if A.P.L (anterior-pituitary-like hormone) is given to very young rats (6-8 days old) no follicle maturation or corpus luteum formation occurs, but the thecal cells increase in size and store large amounts of fat, so that their appearance becomes very similar to that of real corpus luteum cells. Normal reactions to A.P.L—that is, follicle maturation and corpus luteum formation—is regularly found in prepubertal rats if the injections are started around the 21st day of life. We found, however, that rats treated daily with this hormone, beginning at the age of 6 days, react with thecal luteinization only, even when they reach the age of 21 days or more.[1]

In the present experimental series, we wished to see (1) if the formation of thecal lutein cells precedes the appearance of the squamous vaginal reaction and, (2) how long these rats continue to react to A.P.L. with exclusively thecal luteinization and continuous oestrus. For this purpose, 43 female rats were injected with increasing amounts of A.P.L. (6-25 units daily), beginning at the age of 6 days. We examined the vaginal smears every day and killed an animal every few days in order to study the histological changes produced in the ovary. These experiments showed that at the time when the smears first became oestrous—on the seventh to the thirteenth day of treatment—the formation of thecal corpora lutea was already very conspicuous. All the animals killed during the next 4 to 5 weeks showed a similar reaction, inasmuch as in all of them only thecal cells turned into lutein cells. As the treatment progressed, severe follicular atresia set in, so that most of the primary follicles disappeared and histological preparations in these stages showed almost nothing but small islands of luteinized thecal cells. In some cases inconspicuous remnants of the granulosa or the ovum could be detected on serial sections in the middle of these islands, but later even these traces disappeared. The reason for this severe follicular atresia is yet unknown, but considering that the granulosa has no blood vessels of its own, and is therefore entirely dependent upon the theca for its nutrition (as is the ovum itself) the disappearance of these parts of the follicle may simply be the result of the changes produced in the theca.

Between the thirtieth and fortieth day of treatment the oestrus became discontinuous and irregular cycles appeared. The ovaries examined at this stage of the experiment showed several mature follicles and real corpora lutea in various stages of development. These corpora lutea were undoubtedly formed from maturing follicles, the granulosa cells of which became luteinized. At the same time the thecal corpora lutea disappeared. At this stage of our experiment some new factor must have appeared which enabled A.P.L. to produce its normal effect on the ovaries.

According to our conception of the mechanism of A.P.L action[2, 3] the factor responsible for the change in the reaction of the ovary to this hormone at this stage must be a complementary substance furnished by the pituitary. In hypophysectomized animals one finds thecal luteinization only[4] unless suitable pituitary extracts are given together with A.P.L.[2, 3] It is most likely that the immature pituitary is unable to furnish the complementary substance necessary for the normal action of A.P.L on the ovary, but it becomes able to do so before normal puberty is reached, and at this time A.P.L. will produce precocious puberty.

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[1] Selye, H., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 647.

[2] Collip, J. B., Selye, H., and Thomson, D. L., *Virchow's Arch.*, 1933, in press.

[3] Collip, J. B., Selye, H., Thomson, D. L., and Williamson, J. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 590.

Many animals do not react as well to A.P.L as the rat does. Jares[5] stated that intravenous administration of pregnancy urine fails to induce ovulation in adult virgin guinea pigs, nor could he detect any histological changes in the ovaries of these animals. As it is impossible to induce a normal ovarian reaction in the guinea pig, the effect of A.P.L has not been studied adequately in this species. Thinking that the reason for this lack of reaction might be the inability of the guinea pig's pituitary to furnish the complementary substance necessary for A.P.L action, we studied the histological effect of this hormone on the ovary more closely in order to deter-

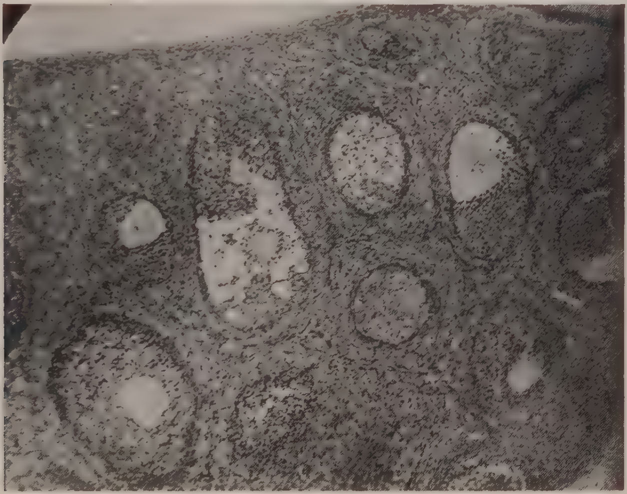

FIG. 1.

Ovary of guinea pig weighing 126 gm. treated daily for 6 days with 300 units of A.P.L. Picture shows proliferation of thecal cells which assume the appearance of lutein cells.

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[4] Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

[5] Jares, J. J., *Anat. Rec.*, 1931, **49**, 185.

mine whether changes would appear similar to those seen in the hypophysectomized rat—that is, thecal luteinization.

Six virgin guinea pigs, weighing 136-321 gm., received various amounts of A.P.L. (200-700 units per day for 6-14 days); the ovaries of these animals were enlarged, but showed no signs of follicle maturation or corpus luteum formation. The thecal cells, however, had changed into luteal cells.

These experiments seem to support the conception that species differences in the reaction to A.P.L may be due to differences in the ability of the pituitary to furnish the complementary substance necessary for the normal reaction of A.P.L. on the ovary.

6672

A Lactation Hormone of the Adrenal Cortex.*

K. A. BROWNELL, J. E. LOCKWOOD AND F. A. HARTMAN.

From the Department of Physiology, University of Buffalo.

One of us[1] showed that adrenalectomized rats could bear young if treated with cortin. No attempt was made to raise the young. Carr[2] found that cortical extract made according to the Swingle and Pfiffner method did not support lactation. Swingle and Pfiffner[3] have recently shown that their extract will support lactation in the dog.

Extract made according to our method which removes fatty substances by chilling to —12°C. does not enable adrenalectomized rats to supply milk (B, Table I) even when used in large amounts (C), yet it is potent as far as the cortin content is concerned. On the other hand, if the chilling is not carried below 3°C., our extract supports lactation (D). The material chilled out between 3 and —12°C. contains a substance necessary for milk production, since if an extract of this material is added to cortin and injected into adrenalectomized mother rats, they raise a much larger proportion of their young (E).

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* Aided by a grant from the Carnegie Corporation of New York to the Carnegie Institution of Washington.

[1] Hartman, F. A., *Endocrin.*, 1930, **14**, 229.

[2] Carr, J. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 131.

[3] Swingle, W. W., and Pfiffner, J. J., *Med.*, 1932, **11**, 371.

TABLE I.
Effect of Cortical Extracts on Lactation.

	Daily Injections	No. Mothers	Young Born	Young Reared	% Reared
A	Stock animals	27	157	113	72
B	Cortin 2 cc. of 1-40	9	42	1	2
C	Cortin 2 cc. of 1-80	4	30	4	13
D	Cortical extract 2 cc., 1-80	13	89	57	64
E	1 cc. lactation hormone + 1 cc. cortin (1-30)	5	33	17	51

The evidence indicates the existence of a new hormone in the adrenal cortex which supports lactation. *Cortilactin* is the name suggested for this new hormone.

6673

Further Experiments on Induction of Ovulation in Toads.*

I. A. WILLS, G. M. RILEY AND E. M. STUBBS. (Introduced by E. Witschi.)

From the Zoological Laboratory, State University of Iowa.

The authors have been interested in the problem of specificity of the maturity hormone of the anterior lobe of the hypophysis. Previously we[1] have reported that heteroplastic hypophysis implants from frogs may be used to induce ovulation in toads, thus proving the non-specificity of the maturity hormone in anurans. Kuyper, Pfeiffer and Wills,[2] on the other hand, were unable to produce ovulation in toads using injections of hebin (prepared from pregnancy urine). In our present experiments, we wished to further test the specificity of the hormone by implanting into toads, hypophyses from 2 different vertebrate classes. Garpike and albino rats were used.

A series of 6 *Bufo americanus* females were given daily implants of 2 to 4 hypophyses from garpike *(Lepidosteus platystomus Rafinesque* and *L. osseus L.)*. Ovulation occurred in 5 animals between the second and sixth day. The sixth female died of an infection without ovulating. The best results were obtained by implanting 4 hypophyses daily. The discharged eggs in some cases appeared to be overripe. In several cases all the eggs were not discharged at one time. In most cases the eggs were retained in the uteri longer than normally. Males placed with the females did not copulate and artificial fertilization was not attempted so the eggs did not develop.

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Emil Witschi.

[1] Wills, I. A., Riley, G. M., and Stubbs, E. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 411.

[2] Kuyper, A. C., Pfeiffer, C. A., and Wills, I. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 413.

One "jumbo" female frog *(Rana pipiens)* was given daily implants of 4 Lepidosteus hypophyses. Eggs were laid on the fourth day. Ovulation and eggs appeared normal.

Two toads were given daily implants of 2 and 4 hypophyses from albino rats for 5 and 8 days respectively. Ovulation did not occur. The animal which received 2 hypophyses daily, died at the end of the fifth day as a result of shock and hemorrhagic condition in the region of the implants. The other female received 2 glands daily for 4 days and 4 glands daily for an equal period. This animal died a month later. Autopsy of both females showed that the ovaries were filled with normal eggs.

Evidence is accumulating that ovulation in all vertebrates is controlled by a mechanism in which the hypophysis plays a major rôle. Houssay[3, 4] has reported ovulation in fish, amphibians, and reptiles (snakes) after homoplastic implants had been made. In birds and mammals, various workers have shown an interrelationship between the hypophysis and the gonads but just what rôle the hypophysis plays, directly or indirectly, in the process of ovulation has not been determined with certainty. It may be that the maturity hormone is non-specific as are other hormones, or it may be specific in certain groups of animals. Houssay and associates,[4, 5] as a result of the failure to secure ovulation in toads using hypophysis implants from hen, rat, guinea pig, dog, cattle, frog, snake, and fish believe that there exists a "zoological specificity" of the hormone. Our previous experiments in which we obtained ovulation in toads, using frog hypophyses, and the present experiments, using fish, show that part of their conclusions are erroneous but the results of our experiments using rat hypophyses are in agreement with theirs. Examination of their data indicates that probably they did not use large enough numbers of hypophyses to secure positive results in the case of frog and fish implants. The failure to obtain ovulation using implants from rats may be due to a specificity between the groups or

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[3] Houssay, B. A., *Compt. Rend. Soc. Biol.*, 1931, **106**, 377.

[4] Houssay, B. A., Giusti, L., Lascano-Gonzalez, J. M., *Compt. Rend. Soc. Biol.*, 1929, **102**, 864.

[5] Houssay, B. A., Giusti, L., Lascano-Gonzalez, J. M., *Rev. Soc. Argent. Biol.*, 1929, **5**, 397.

to a destruction of the hormone of the implanted glands by the host as a result of the incompatibility of the tissues and the subsequent reactions. The results of the present experiments only suggest that the maturity hormone among lower vertebrates is non-specific.

6674

Demoor's Active Substance of the Heart.

C. H. MC DONALD AND A. C. MC DONALD. (Introduced by W. H. Howell.)

From the Department of Physiology and Pharmacology of the University of Arkansas, School of Medicine, Little Rock.

Demoor[1, 2] has described a humoral regulatory substance elaborated by the nodal tissue of the heart which has the property of conferring rhythmicity upon the aperiodic beat of an isolated left auricle. His theory is based chiefly upon the observation that the right auricle of the rabbit or guinea pig, excised and dropped into a Ringer-Locke solution, soon assumes a regular rhythm: the left auricle, however, gives only aperiodic, jerky beats in this solution. If now, the solution in which the right auricle has been active for a while is poured over the left auricle, the latter gives rhythmical beats. Demoor has prepared both saline and alcoholic extracts of the right auricle of the rabbit which were able to confer periodicity upon the isolated left auricle of the rabbit. He has named this unknown substance the "active substance" of the heart. The writers have been able to confirm his observations.

All solutions used in the experiments were made from Baker's analyzed chemicals, recrystallized. The Ringer-Locke solution employed consisted of $NaCl$ 0.9%, $CaCl_2$ 0.024%, KCl 0.042%, $NaHCO_3$ 0.01%, and Dextrose 0.1%. The pH was adjusted to 7.4, and the solution was oxygenated and maintained at a temperature of 37°C. throughout the experiment. An adult rabbit was placed under light ether anesthesia, the heart was exposed and perfused through the ascending vena cava with the Ringer-Locke solution for a short time to free the heart of blood. The heart was then excised and both auricles removed, care being taken to see that the venous

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[1] Demoor, M. J., *Annales de Physiol. et de Physico-Chimie Biol.*, 1929, **5**, 1.

[2] Demoor, M. J., and Rylant, M. P., *Bull. Acad. Roy. de Med. de Belgique—Seance*, Oct. 26, 1929, 585.

openings were included in each preparation and that the left auricle was wholly free of septum. Each auricle was then placed in a few cc.'s of the warmed Ringer-Locke solution. In most cases the right auricle gave rhythmical beats and maintained them for varying lengths of time. In no case did the left auricle exhibit rhythmical beats although a few aperiodic beats sometimes occurred. If the right auricle continued beating for 20 to 30 minutes, pouring of the solution, in which it had been active, over the left auricle, which had remained inactive in its Ringer-Locke solution, was followed in 70% of our trials by the assumption of rhythmic beats by the left auricle. Neither histamine nor adrenalin conferred rhythmicity upon an inactive left auricle. The quiescent left auricle of the white rat also assumed rhythmicity when placed in the Ringer-Locke solution in which a right auricle of the rabbit had been allowed to beat, or when treated with a saline extract of the right auricle of the rabbit. The period during which any left auricle in this series of experiments continued beating was never over 10 minutes.

Saline extracts were made of the 2 auricles according to the following method. A rabbit was operated upon in the manner described above, except that perfusion was done with a 0.9% solution of recrystallized sodium chloride. The isolated auricles were slit open and washed free of blood by gently rubbing them with the rounded end of a fire-polished glass rod. They were then shaken free of the solution and roughly weighed. The auricles were ground to a paste with glass wool which had been boiled in redistilled water and washed repeatedly with the same. The paste was then made up to approximately 50 cc. with 0.9% sodium chloride solution, allowed to stand for 2 hours, then centrifuged at high speed for 15 minutes. The liquid was then decanted and centrifuged twice more for like periods of time. The resulting extract was again made up to 50 cc. with the sodium chloride solution. Each extract was tested for its ability to confer rhythmicity upon the inactive auricle of the white rat before subjecting it to any chemical examination. In all, 12 extracts were examined. Identical tests were conducted upon extracts of the right and left auricles. The extract of the right auricle invariably showed a higher degree of opalescence than did that of the left auricle. A definite but very thin film was always deposited upon the container of the extract of the right auricle which was absent in the extract from the left auricle. Both the substance responsible for the greater opalescence of the extract from the right auricle and this film were soluble in ether and carbon disulphide. Quantitative examination of these extracts for calcium and potassium showed no difference in results for the 2 auricles. The average for calcium was 0.3 mg. per gram of tissue for the right auricle and 0.28 mg. for the left auricle. The average for potassium was 2.8 mg. for the right auricle and 2.71 mg. for the left.

Tests for nitrogen and sulphur (lead acetate reaction) were negative, as also were Nylander's test, Bial's test, Tollen's test, and the reduction of picric to picramic acid. Fehling's solution and the Shaffer-Hartman test showed slight reduction, equal in the 2 extracts. Both extracts reduced methylene blue and gave a positive test to the Molisch reaction.

6675

Antigenic Structure of Certain Chlorellas and Allied Forms.

T. D. BECKWITH.

From the Department of Bacteriology, University of California, Berkeley, and Laboratory of Bacteriology, University of California at Los Angeles.

A series of tests has been conducted in an endeavor to determine whether there is an antigenic protein fraction common to a number of related unicellular algae. The following forms have long been held in culture within our collection: *Chlorella variegata, Chodat; Chlorococcus humicola (Naeg) Rabenhorst; Mannochloris bacillaris, Neumann; Chlorella viscosa, Chodat; Chlorella vulgaris, Beij, var. genevensis, Chodat; Chlorella rubescens, Chodat; Chlorella luteo-viridis, Chodat.* In addition there was available another undetermined species of *Chlorella.*

Each one of the algal forms included in this list was grown upon an agar medium of the following composition: Magnesium sulphate, 0.25 gm.; calcium nitrate, 1.0 gm.; monobasic potassium phosphate, 0.25 gm.; potassium chloride, 0.12 gm.; ferric chloride, trace; distilled water, 1000 cc. This solution was now diluted threefold with distilled water and to it in turn was added 1½% agar. After sterilization, the forms were grown upon slants of the medium. Incubation proceeded in diffuse daylight at room temperature over a period of 3 weeks. The resulting growth of each organism was suspended in sterile physiological saline and then with no further treatment each antigen was injected into the posterior aural vein of a rabbit. No signs of toxicity appeared in any one of the animals as the result of this treatment. Immunization progressed by means of 9 injections extending over a period of 18 days. The amount of suspension introduced commenced with 0.25 cc. and the last 5 were each of 1 cc. amounts. There was no evidence of anaphylaxis during the period of this sequence of injections. Trial bleedings made during the latter part of this immunizing treatment showed the presence of agglutinins and the usual titre computed by 2 hours incubation at 37°C. followed by storage at ice box temperature was 1:320 with complete clearing of the tubes.

When immunization had progressed to the desired point, the animals were exsanguinated, the serum was separated by centrifugalization following defibrinization and the resulting preparations were then stored without addition of preservative until used. Usual titrametric methods were followed in setting up the agglutination series and 2 controls were added. These checks consisted of the antigen suspended in physiological saline and of the antigen placed in contact with 1:25 normal rabbit serum.

These experiments dealing with agglutinin mobilization showed that each antigen was clumped by its own homologous antiserum. No cross agglutination whatever appeared. Thus each antigen was agglutinated by its homologous antiserum and by no other. It was thus demonstrated that the protein content of these unicellular algae is antigenic. There is much complexity in the antigenic structure of these forms however for there is no antigenic fraction which is common to the entire series of genera and species.

6676

Quantitative Character of Coccidian Infection in Recipients of Blood from Immunized Animals.*

E. R. BECKER AND P. R. HALL.

From the Iowa State College.

The writers have shown[1] that rats may be immunized to the coccidium *Eimeria miyairii* by feeding each day for 5 days or less standard doses of sporulated oocysts. Other workers have demon-

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* The work leading to the results herewith reported was supported by a grant from the Rockefeller Fluid Research Fund at Iowa State College.

[1] Becker, E. R., Hall, P. R., and Hager, A., *Iowa State College J. Science,* 1932, **6**, 299.

strated the development of a specific immunity in coccidiosis of certain other hosts. The nature of the immunity, however, still remains problematic. In the present experiment injections of blood from hyperimmunized animals were employed in the attempt to reduce the number of oocysts eliminated by the recipients to below the normal.

Nineteen Wistar A rats and 9 rats from a random strain were each given in all from 6 to 9 cc. of citrated or defibrinated blood (not including the citrate solution), some intravenously, some intraperitoneally. The blood injections for each rat were made on either 2 or 3 occasions, and in most cases were planned so that one injection would come from 1 to 9 days previous to the date of the first infective dose, and the others on the day of the first infective dose and several days later. Each rat was infected with 1500 oocysts daily for a period of 5 days, and all the oocysts passed during the period of elimination were conserved and counted by a dilution method. For each recipient of immune blood a litter mate of about the same weight was kept as a control. The controls received the same immunizing doses of parasites and other treatment as the rats receiving blood, except that they were not injected.

The mean number of oocysts eliminated by the Wistar A series receiving immune blood was $176.2 \pm 14.73 \times 10^6$ oocysts, while the controls eliminated $161.5 \pm 8.063 \times 10^6$ oocysts. The ratio of the difference of the means to the probable error of the difference is 0.8, an insignificant number. The random series receiving blood eliminated an average of $61 \pm 5.4 \times 10^6$ oocysts, while the controls eliminated an average of $50.4 \pm 3.7 \times 10^6$ oocysts. The ratio of the probable error to the difference of the means in this case is 1.6, an insignificant number.

The experiment, which involved 28 rats in the experimental series and 28 in the controls, showed that the blood from hyperimmunized rats contained no principle capable of inhibiting in the least the numerical increase of the parasite in the rat into which it was injected, and that the somewhat lower mean yields in the control series may be attributed to chance.

6677

Chest Width-Hip Width Index.*

ROWLAND G. FREEMAN, JR.

From the Institute for Child Guidance, New York.

In the human skeleton the relationship between chest width and hip width is constantly changing throughout the period of development from birth to maturity. At birth the chest width exceeds the hip width in both males and females. This difference increases during the first 18 months of life due to the higher growth rate of the chest width. During the period from 18 months to 2½ years the rate of growth of the chest width is slowed down and that of the hip width is accelerated, resulting in the 2 measures becoming equal between 3½ and 4½ years. The chest width thereafter continues to grow at a slower rate than the hip width up to the age of puberty.

During the 7th year the growth rate of the hip width in the majority of females is more rapid than in the males. Thereafter hip width in the female exceeds that in the male and at the tenth year another spurt on the part of the female increases the difference. In order to express this changing relationship between hip and chest an index was used derived by dividing the chest width at any given age by the hip width. It was found that this index is above 100 in both males and females up to the fourth year, decreasing thereafter

TABLE I A—Boys
Average Chest Width-Hip Width Index from Birth to 15 Years.

Age	f	Index	S.D.
1 day	160	108.22	6.94
12 mos.	18	113.07	6.75
24	33	110.08	5.56
36	58	105.07	5.07
48	36	101.99	5.22
60	35	98.41	5.30
72	39	96.21	5.85
84	55	95.77	6.25
96	30	95.59	3.04
108	30	94.45	5.11
120	31	93.21	5.29
132	27	93.38	5.13
144	34	92.82	6.64
156	31	93.23	5.30
168	23	93.46	4.13
180	10	95.65	5.08

TABLE I B—Girls
Average Chest Width-Hip Width Index from Birth to 12 Years.

Age	f	Index	S.D.
1 day	149	109.10	6.96
12 mos.	30	111.51	7.04
24	37	110.62	7.78
36	49	105.96	4.92
48	31	100.95	6.95
60	35	96.54	6.74
72	38	94.90	5.04
84	39	94.16	4.41
96	37	94.50	4.55
108	27	92.52	5.44
120	18	90.06	4.42
132	11	89.10	4.60
144	12	88.67	2.48

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* Material collected at Child Development Institute, Teachers College, Columbia University.

to reach a figure at the twelfth year of 88 in the female and 94 in the male. Tables I-A and I-B show the averages of the chest width-hip width index in both boys and girls at each age.

A special study is being made of those children who had a higher index than the average for their age and those who had a lower index than the average for their age. Present findings indicate that girls who menstruate earlier than 12½ years have an index below the average, and that those girls who menstruate later than this age have, in the majority of cases, an index greater than the average. Onset of maturity in boys is more difficult to diagnose. It was found, however, that all the obese boys with retarded genital development had an index above the average for their age.

6678

Vasomotor Responses of Mucosa of Upper Respiratory Tract to Thermal Stimuli.

IRWIN G. SPIESMAN. (Introduced by Lloyd Arnold.)

From the Department of Ear, Nose and Throat, College of Medicine, University of Illinois.

Chilling of the cutaneous surface was assumed in the past to be associated with congestion of the mucous membrane of the nose and throat and that this congestion was favorable to bacterial invasion. The reverse have been the findings in the experiments conducted. A cold stimulus applied to the cutaneous surface produces a reflex vaso-constriction and a lowering of the temperature of the mucosa of the upper respiratory tract, and a hot stimulus produces a reflex vaso-dilatation.

One hundred and sixty-four experiments were performed and distributed as follows: 81 general experiments on one normal man, 52 experiments on 8 normal men, 31 experiments on 15 hyperesthetic rhinitis cases including 3 children. These experiments were conducted in a regular laboratory room with a temperature varying between 21° and 26°C., and in which drafts were minimized. The thermo-electric method was employed with a thermopile electrode applied to the mucosa of the nose and the E. M. F. measured through a potentiometer. Cold air, cold sprays, and aluminum cups, varying in diameter from 4 to 19 cm., partly filled with chipped ice were used as cold stimuli. The ice filled cups gave a skin temperature of 12.60°C. The cups were applied to the upper and lower dorsals and to the lumbo-sacral regions of the back, to the upper and lower anterior chest, to the upper and lower abdomen, and to the feet.

The findings were: I. *Normal Reactions.* (a) The 4 cm. ice cup was the smallest cold stimulus to produce a reflex lowering of the temperature of the mucosa of the upper respiratory tract. (b) The normal response to a short circumscribed cold stimulus (2 minutes) was first a lowering of the mucous membrane temperature, then a rise and gradual return to the initial temperature. (c) The normal response to a continuous circumscribed cold stimulus (10 minutes) to one cutaneous area or to numerous cutaneous areas applied in regular succession, *i. e.*, when the reaction was completed in one area, the stimulus was immediately removed to another, was first a lowering of the mucous membrane temperature and then a gradual rise, stopping short in the majority of the experiments from reaching the initial temperature by 0.18 to 0.36°C.

It will be noted here that with a continuous cold stimulus the thermal endings in the skin gradually "adapted" themselves to this stimulus. "Adaptation" is a process associated, to a certain extent, with the phenomenon of fatigue. Whether this continuous cold stimulus which produces a reflex vaso-constriction, first of maximal and then minimal degree, is a phenomenon of "adaptation" or fatigue is being determined by further experimentation.

(d) A cold stimulus applied to an anesthetized cutaneous area, in which pain was removed, produced a reflex lowering of the mucous membrane temperature. Since thermal impulses take a course similar to pain this proved that pain is not a factor in producing reflex vaso-constriction. (e) Changes in the temperature of the mucosa of the upper respiratory tract to cold stimuli were proved to be the result of a reflex change in the vaso-motor tone of the vessels supplying the mucosa. A fall in temperature indicated a vaso-constriction, a rise, a vaso-dilatation.

II. *Abnormal Reactions.* (a) Head colds (infections). Prior to,* during and following a head cold, there is a period of vaso-motor suspense, *i. e.*, a cold stimulus applied to the cutaneous surface produces no vaso-motor response in the mucous membrane. This suspense of the vaso-motor reaction is unquestionably of a local effect within the mucosa, produced by the toxic products of the infection upon the vaso-motor endings in the mucosa. (b) Hyperesthetic Rhinitis. The hyperesthetic cases, using the same method as in normals, produced erratic vaso-motor changes. Frequently in such cases a cold stimulus on the cutaneous surface produced a reflex rise in the mucosa temperature instead of a drop; a hot stimulus produced a lowering of the mucosa temperature instead of a rise.

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* On several normal subjects daily experiments were made. In some of these during April and December, 1932, head colds developed. It was found in these subjects that the day prior to the actual appearance of the signs and symptoms of a head cold and during the head cold there was a vaso-motor suspense.

6679

Thyroid Hypertrophy as a Response to the Gonad-stimulating Hormone of the Pituitary.

OSCAR RIDDLE, ROBERT W. BATES AND SIMON W. DYKSHORN.*

From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Following our[1] isolation of a third anterior pituitary hormone (prolactin) in fairly pure form, together with the purification of the growth and gonad-stimulating hormones facilitated by this accomplishment and notably by the very recent purification of the growth hormone,[2] it is perhaps admissible to attempt the association of certain hitherto observed structural and functional responses with each of the (3) now known hormones of the anterior pituitary. If still other now unrecognized hormones exist in the pituitary this attempt may be premature. In our opinion, only accelerated body growth was hitherto a proved specific response to the growth hormone; only accelerated gonad growth a proved specific response to the gonad-stimulating hormone; and only activation of crop-glands and lactation proved specific responses to prolactin. Many other responses are known but remain unassigned to a specific pituitary hormone. On the basis of scant evidence a few previous workers have variously suggested that thyroid hypertrophy is a response to the growth, the gonad-stimulating and to a specific thyreotropic hormone.

The data tabulated here are taken from several hundreds of assays of pituitary extracts or fractions (in very various stages of hormone isolation and purification) injected into doves and pigeons. These animals afford an exceptionally quick and easy test for prolactin, probably a fair test for the growth hormone, and the immature males are probably the most sensitive known test for the gonad-stimulating hormone. The thyroid response can be studied to some advantage in our colony of animals, since various races have here

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* Deceased.

[1] Riddle, O., Bates, R. W., and Dykshorn, S. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1216. (Also, *Am. J. Physiol.*, 1933, in press.)

[2] Collip, J. B., Selye, H., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 544.

TABLE I.

Thyroid hypertrophy in ring doves is obtained from preparations which contain the gonad-stimulating hormone. Growth hormone and prolactin, when free of the gonad-stimulating principle, do not stimulate the thyroid.

Ant. pituitary; known contamination	No.	Daily dosage		Body wt. change	Age	Thyroid Test	Thyroid Control (an av.)	Crop gland active (+) or not (—)
		mg.	days	gm.	mo.	mg.	mg.	
Growth (no maturity, trace prolactin) (Collip, Selye, and Thomson)	Q16	1.5	6	147— 3	2.3	13.2	13.8	—
		1.5	8	139+12	3.0	17.0	15.6	+
	Q21	1.5	7	141— 1	2.9	20.1	15.1	+
		1.5	7	147+11	2.3	9.6	13.8	+
Growth (+ maturity) (Lee and Schaffer)	36	1.5*	5	139+ 5	3.1	21.9	14.8	—
		1.5	5	110+ 3	3.0	42.0	20.0	+
		2.2	9	137+ 9	3.2	65.9	20.0	—
		2.2	10	142+28	3.3	36.5	14.8	—
Growth (+ maturity) (traces prolactin) Phyone (Van Dyke)		4	6	172+10	14.0	41.5	15.3	+
		4	7	160+14	14.0	41.7	15.3	+
		8	5	137+ 1	2.8	29.3	13.9	+
		4	8	150+23	2.8	44.3	15.3	+
	36a	?	5	134— 7	2.8	26.4	20.3	—
		?	5	144— 8	2.8	28.4	20.3	—
Gonad-stimulating (+ traces pituitrin) (own preparations)	43	3.5	5	143— 4	2.9	43.9	20.0	—
		3.5	5	143—13	2.9	72.9	20.0	—
		3.5	5	160—12	2.7	24.7	15.3	—
		3.5	7	172— 6	13.7	60.8	13.9	—
	56	2.2	6	157—16	2.9	29.2	20.1	—
		2.2	6	160—16	3.3	52.0	(23.0)	?
	71[1]	3.0	5	160—20	2.2	15.7	16.4	—
		3.0	5	158—24	2.3	12.1	16.9	—
	34	2.5	6	161+ 0	12.2	15.8	17.9	+++
		10.0	5	154— 3	12.1	14.2	17.9	+++
Prolactin (no maturity)	69	10	7	137— 3	3.1	10.7	20.0	++++
		10	7	152—15	2.9	13.5	(11.9)	++++
	93	20	8	159— 2	2.5	18.6	18.1	++++
		20	8	137— 6	3.1	12.4	14.5	++++

* These values (protein) calculated from the nitrogen content, except values for prolactin.

[1] Method of Fevold, Hisaw and Leonard.

been established on the basis of thyroid size[3]; in the present study, however, much of this advantage has been lost because we have been forced to use numerous hybrids of these races, and such hybrids often show a wide variability of thyroid size. Thyroid hypertrophy is measured here solely in terms of increased weight.

The data of Table I, like practically all of the assays made by us, clearly indicate that neither the growth hormone nor prolactin—when these are freed of the gonad-stimulating or sex maturity principle—has any special capacity to cause enlargement of the thyroid. Growth preparations Q16 and Q21, kindly supplied us by Dr. Collip, like our own prolactin preparations, have shown no gonadotropic response whatever when used in the quantities indicated here. The growth preparations of Lee and Schaffer and of Van Dyke and Wallen-Lawrence do contain the sex maturity hormone in addition and these preparations regularly produce thyroid hypertrophy. Our own preparations of the gonad-stimulating hormone, made with somewhat variable procedures, have been found to be free of prolactin when tested in the quantities used here; they probably contain but little growth hormone; they regularly induce rapid growth in the immature male testis and in most cases produce thyroid hypertrophy. Several tests made with excellent gonad-stimulating fractions, prepared from whole sheep pituitary according to the method of Fevold, Hisaw and Leonard,[4] completely failed to increase the weights of thyroids.

The last column of the table provides the test for the presence or absence of prolactin in the various preparations; the maximum prolactin effect is observed only after 7 or 8 days of dosage. The thyroid response, or lack of response, clearly does not depend upon the quantity (apart from quality) of protein injected. Equivalent results, though less satisfactory because of greater thyroid variability, have been obtained on a wholly different species—common pigeons.

Summary. For the first time it is shown that 2 anterior pituitary hormones—prolactin and the growth principle—do not cause the thyroid hypertrophy which characteristically follows the injection of various pituitary extracts. Hyperplasia of the normally developed thyroid following pituitary administration is a specific response to the gonad-stimulating hormone, or to another pituitary derivative having very similar solubilities. Good gonad-stimulating preparations do not invariably induce an increase in thyroid weight in doves and pigeons.

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[3] Riddle, O., *Am. Nat.*, 1929, **63**, 385.

[4] Fevold, Hisaw and Leonard, *Am. J. Physiol.*, 1931, **97**, 291.

6680

Monophasic Electrical Response Produced by the Contraction of Injured Heart Muscle.

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The electrical responses produced by injured heart muscle are of great interest not only because of their important bearing upon the interpretation of the electrical phenomena exhibited by excitable tissues generally, but because of their relation to the electrocardiographic changes that follow coronary thrombosis in man. We here report briefly experiments relating to the electrical responses produced by the injured heart beating *in situ.*

When one of 2 non-polarizable electrodes connected to the terminals of the string galvanometer is placed in contact with a burned region on the epicardial surface of the exposed turtle's heart and the other in contact with the subcutaneous tissues at a point distant from the heart, the ventricular complex of the curve recorded is essentially monophasic in outline. The monophasic character of the response is due to the disappearance during systole of the injury current flowing during diastole. The direction of the deflection indicates that during ventricular systole the cardiac or exploring electrode is relatively positive with respect to the distant or indifferent electrode. Curves of the same type are obtained by leading from the exposed and freshly injured surface of the mammalian auricle or mammalian ventricle to a distant point. In all such experiments we have connected the electrodes to the galvanometer in such a way that relative negativity of the exploring electrode yields an upward deflection in the completed record. In the curves obtained by leading from an injured to a distant region, ventricular systole is, therefore, represented by a monophasic deflection directed downward.

If a monophasic response is obtained, in the manner described, from the ventral surface of the turtle's heart by means of an electrode in which contact with the heart is made by a wick that extends 3 or 4 mm. beyond the insulated shaft of the electrode, flooding the heart with normal saline or with Ringer's solution so that the surface is immersed to a depth of 5 or 10 mm., greatly reduces the amplitude of the curve but does not materially alter its form. If the electrode is insulated to the tip the reduction in amplitude is very much less and may not exceed 50%. The form of curves obtained from the exposed heart is not therefore dependent upon the fact that the region from which the lead is made is bounded by a dielectric.

If the exploring electrode is placed on the heart at a considerable distance from the burned area the curve may be identical with that obtained from the same region before the injury was produced. In the great majority of instances, however, there is a very conspicuous upward displacement of the *RS-T* segment of the ventricular complex, and this displacement is often so pronounced that the curve is almost monophasic in character. In this case, however, the direction of the deflection indicates that the exploring electrode becomes relatively negative with respect to the distant electrode during ventricular systole.

We have pointed out[1] that in leads of the type employed the potential variations of the indifferent electrode are insignificant in comparison with the potential variations of the exploring electrode. The curves obtained are, therefore, essentially records of the potential variations produced by the heart beat at the point in contact with the latter. The monophasic responses obtained by leading from a burned region are consequently due to potential variations that occur within this region in response to electrical forces developed in its immediate neighborhood. The monophasic responses obtained by placing both electrodes in contact with the heart, one upon an injured and the other upon an uninjured region, have usually been attributed to potential variations occurring at the uninjured point, the potential of the injured area being regarded as constant throughout the cardiac cycle. This point of view may have advantages in the interpretation of the monophasic responses obtained from small strips of excitable tissue removed from the body and suspended in moist air. It is misleading when applied to curves obtained from the heart *in situ*. In the latter case the monophasic character of the curve obtained by leading from an uninjured to an injured region is due chiefly to potential variations of the electrode in contact with the injured tissue.

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[1] Wilson, F. N., Macleod, A. G., and Barker, P. S., *Am. Heart J.*, 1932, **7**, 305.

6681

Form of the Electrocardiogram in Experimental Myocardial Infarction.

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In a series of experiments still incomplete we have attempted to determine how the changes in the initial and final deflections of the ventricular complex observed in standard and precordial[1] electrocardiograms in clinical coronary thrombosis are produced. In these experiments, performed upon fully anesthetized dogs, we have ligated the anterior descending branch of the left coronary artery or one of its major subdivisions, and have employed direct and semi-direct leads[1] as well as the usual standard leads in studying the alterations in the electrical phenomena associated with the heart beat that this procedure produces. The results of the experiments carried out thus far may be summarized as follows:

Almost immediately after the ligation of a coronary artery the electrical curves obtained by leading directly from the epicardial surface of the region supplied by it become essentially monophasic, and are similar to those obtained when the same portion of the heart's surface is injured by burning or by other means. The direction of the monophasic response is such as to indicate relative positivity of the electrode in contact with the heart (the exploring electrode) or relative negativity of the indifferent electrode, which is placed in contact with the subcutaneous tissues of the right or left hind leg. The displacement from the base line of the final portions of this monophasic curve, which correspond in time to the RS-T segment and T-deflection of the standard electrocardiogram, rapidly diminishes, but does not, apparently, completely disappear for several days.

Twenty-four to 48 hours after coronary ligation, the electrical responses obtained by leading directly from the infarcted region are quite different from those described in the preceding paragraph. They show no proper intrinsic deflection and no preliminary downward movement such as precedes that deflection in the curves obtained from other portions of the heart. They begin with a tall

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[1] Wilson, F. N., Macleod, A. G., and Barker, P. S., *Am. Heart J.*, 1932, 7, 305.

upward deflection, often notched near its apex or on its descending limb, which corresponds in time to the *QRS*-deflections in lead *I*, and indicates by its direction that the exploring electrode is relatively negative during its inscription. The *RS-T* segment which follows this initial deflection is displaced downward and the *T*-deflection is upright. During the period when curves of this type are obtained from the infarcted region, this region, at least when it is large, appears relatively immobile and does not seem to take part in ventricular contraction. Some parts of the infarcted region fail to respond to strong induction shocks; other portions respond but display a considerably prolonged absolute refractory period. At the margins of the infarcted region there appears to be a gradient in the length of the refractory period.

Serial semi-direct leads have been employed in the same manner as in an experimental study of bundle branch block recently published from this laboratory.[2] The curves obtained by this method of leading 24 to 48 hours after ligation of the anterior descending branch of the left coronary artery in the dog are similar in all respects to those obtained by means of serial precordial leads in patients who have had a recent attack of coronary thrombosis and who display standard electrocardiograms of the type attributed to infarction of the anterior surface of the left ventricle.[2] The standard leads also show strikingly similar electrocardiographic changes in dogs and in man.

Averbuck and Rachmilewitz[3] have reported that, after ligation of subdivisions of the circumflex branch of the left coronary artery in the dog, section of the right branch of the His-bundle does not produce characteristic changes of the usual kind in the electrocardiogram. In our experience section of this bundle branch after ligation of the anterior descending branch of the left coronary artery produces electrocardiographic changes that differ only slightly from those produced in normal animals by the same procedure.

These observations strongly support the view that the changes in the *QRS*-deflections of the electrocardiogram that occur in coronary thrombosis are due to the disappearance of the electrical effects normally produced by the infarcted muscle; that the displacement of the *RS-T* segment from the base line is due to the disappearance during systole of a current of injury flowing at the margins of the infarct; and that the curious *T*-wave changes that have attracted so much attention are due to a prolongation of the excited state in the same region.

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[2] Wilson, F. N., Macleod, A. G., Barker, P. S., and Klostermeyer, L. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1006.

[3] Averbuck, S. H., and Rachmilewitz, M., *Z. f. d. ges. Exp. Med.*, 1931, **75**, 562.

6682

Reaction Time and Chronological Age.

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Although the literature dealing with reaction time is enormous, no studies seem to have appeared of the relationship between reaction time and chronological age in a large group of individuals having a wide span of age limits. It seemed desirable that this relationship be studied.

We employed 150 individuals, sampled at random and ranging from the ages of 4 years to 60 years, grouped, for convenience, as shown in Table I.

TABLE I.

Group	Age Limits	Av. Age of Males	Av. Age of Females
	yr.	yr.	yr.
A	4-10	7.3	5.4
B	11-20	18.0	16.3
C	21-30	24.5	23.5
D	31-40	36.1	35.8
E	41-50	44.8	45.5
F	51-60	55.1	58.4

Each group contained 20 individuals, except Group F which had 10. Half the subjects in each group were females.

Our purpose being to determine the reaction times to light and sound, 2 simple arrangements were constructed. For light, an ordinary 50 watt electric light was placed in series with one of the poles of a double throw switch, the other pole being in series with a signal magnet, recording on a moving drum, the operator's key, and the subject's tap key, the response recorded by tapping the key and breaking the circuit. The apparatus for sound consisted of a signal magnet in series with the operator's and subject's tap key placed in the primary circuit of an induction coil, with a set of head telephones in the secondary circuit.

The subjects were always seated with arm and body in the same position and given the simple instructions necessary. The experimenter's operations and recording devices were not visible to the subjects who were told that they would have a number of practice trials before the actual recording was begun. The number of practice attempts was arbitrarily determined by the operator from the nature of the responses; if it seemed advisable to allow more attempts to make the necessary neuromuscular adjustments, it was done. Final calculations were made from the subject's 5 best responses.

Averaging the times for males and females separately in each group (Table II), we find that males always respond more quickly than females, especially in childhood and late maturity, the figures closely approximating each other in the third decade. The reaction time to sound is always less than to light.

TABLE II.

	Male Averages				Female Averages			
Group	Light	S.D.	Sound	S.D.	Light	S.D.	Sound	S.D.
A	.34	.1070	.34	.0928	.62	.1644	.59	.1890
B	.24	.0400	.23	.0409	.32	.0340	.31	.0407
C	.22	.0331	.19	.0338	.26	.0192	.20	.0736
D	.26	.0465	.24	.0141	.34	.0378	.30	.1139
E	.27	.0266	.25	.0467	.36	.0342	.30	.0372
F	.38	.0574	.37	.0806	.44	.0721	.42	.0842

S.D. = standard deviation.

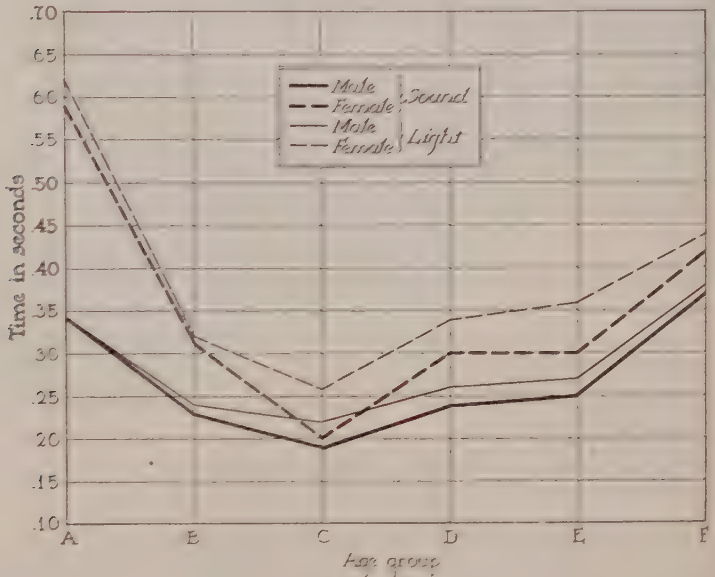

Fig. 1.

It should be recalled from Table I that although each group represents a 10-year span, the average reaction times are those corresponding to the average age of the individuals in a particular decade.

When the results are plotted in the form of a curve (Fig. 1), the striking fact becomes apparent that the shortest times are elicited between the ages of 21 and 30 years (Group C), with decrements approaching from earlier and later age groups. Thus, Rutherford's figures[1] for reaction time to sound as .15-.16 seconds, and to light as .20-.22 seconds would only hold for Group C. Our shortest figures for males show an average time of .22 seconds in response to light, and .19 seconds in response to sound. For females, the shortest average time to light is .26 seconds, and .20 seconds in response to sound.

There is a 73% increase in reaction time to light and a 95% increase in reaction time to sound of the males in Group F over those in Group C. Similarly, there is a 70% increase to light and a 110% increase to sound of the reaction time of females in Group F over those in Group C.

In the determination of reaction time, there are 2 requisites (Johnson,[2]) (a) the adequacy of the stimulus, and (b) the neuromuscular set. To meet requirement (a) for light, a 50 watt lamp was used at a distance of 3 feet from the subject with the current turned on until the response was made, while for sound, the intensity was adjusted for each subject until it was clearly audible, being kept on until the response was made. The neuromuscular set was established by the practice trials preceding the actual recording.

There may be some relation between the curve obtained in Fig. 1 and the parallel curve for mental growth, the latter increasing rapidly until late adolescence, showing a plateau from the late 'teens through the twenties, with the peak at 18 years, and then slowly falling off with approaching senility (Moss,[3] Pintner,[4] Sorenson,[5] Miles and Miles[6]).

It is probable that the great number of accidents among very old and very young persons is related to their longer reaction times.

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[1] Rutherford, *Proc. Roy. Soc. Edin.*, 1893, **20**, 328.

[2] Johnson, H. M., *Psych. Bull.*, 1923, **20**, 562.

[3] Moss, F. A., "Applications of Psychology," Houghton, Mifflin Co., Boston and New York, 1929, 158.

[4] Pintner, R., "Intelligence Testing," H. Holt and Co., New York, 1931, 86.

[5] Sorenson, H., *J. Educ. Psych.*, 1930, **21**, 451.

[6] Miles, C. C., and Miles, W. R., *Am. J. Psych.*, 1932, **44**, 44.

6683

Studies in Cancer: The Application of the Rupp-Schied-Thiel Thiocyanate Reaction to the Urine.*

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Saxl[1] concluded that quantitatively thiocyanate is increased in cancer with values appreciably higher than normal and higher than found in any other disease. He employed the Rupp-Schied[2] method as improved by Thiel.[3] Making use of the same method we found great variation in the apparent thiocyanate content of both normal and pathological 24-hour urines. A number of cases of marked cancer involvement showed high apparent thiocyanate as found by Saxl but other marked cases of cancer were within normal limits and sometimes below normal. It would seem that the excretion of material behaving like thiocyanate is not necessarily increased in cancer. The average in cancer is somewhat higher than for other pathological conditions tested by us since some cases as, for example, multiple myeloma, were much higher than normal. The values found in our cancer cases show that irrespective of what the Rupp-Schied-Thiel thiocyanate procedure is measuring when applied to urine, thiocyanate or other similarly reacting material is not necessarily increased in cancer.

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* This work was supported by a Research Grant from the Chemical Foundation.

[1] Saxl, P., *Biochem. Z.*, 1913, **55**, 224.

[2] Rupp, E., and Schied, A., *Ber. Chem. Ges.*, 1902, **35**, 2191.

[3] Thiel, A., *Ber. Chem. Ges.*, 1902, **35**, 2766.

6684

Evaluation of the Rupp-Schied-Thiel Method as a Test for Thiocyanate in the Urine.*

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While applying the Rupp-Schied-Thiel[1, 2] thiocyanate procedure to pathological urines, cancerous, and non-cancerous and to normals, as given in the preceding paper, the question arose as to the degree of specificity of the reaction when applied to a number of substances known to occur in urine or possibly there. Among the substances possibly in urine found to behave like thiocyanate in the Rupp-Schied-Thiel procedure are ergothioneine, urinary proteose, and oxyproteic acids, and to some degree uric acid. Four normal urines were studied for total apparent thiocyanate by the Rupp-Schied-Thiel procedure; for ergothioneine-like material colorimetrically against blood ergothioneine by the Behre and Benedict[3] procedure; urinary proteose by the method of Oriel and Barber[4]; and for oxyproteic acids as barium salts according to Pregl.[5] In each case the thiocyanate equivalent of the fractions, (1) ergothioneine-like material, (2) proteose, and (3) barium oxyproteic acids was determined in the Rupp-Schied-Thiel procedure. The thiocyanate equivalents of fractions listed subtracted from the total apparent thiocyanate shows much less thiocyanate than given by the Rupp-Schied-Thiel method. The conclusion is drawn that the Rupp-Schied-Thiel method is not a measure of thiocyanate if applied directly to the urine. The same objection holds for the Munk[6] gravimetric method which depends on the precipitation of thiocyanate by silver nitrate and melting the precipitate with sodium carbonate and potassium nitrate to oxidize the sulfur to sulfate, which is then precipitated as barium sulfate. Other urinary sulfur compounds than thiocyanate are included in this method.

The interfering material, however, can be eliminated. Thus,

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* This work was supported by a Research Grant from the Chemical Foundation.

[1] Rupp, E., and Schied, A., *Ber. Chem. Ges.*, 1902, **35**, 2191.

[2] Thiel, A., *Ber. Chem. Ges.*, 1902, **35**, 2766.

[3] Behre, J. A., and Benedict, S. R., *J. Biol. Chem.*, 1929, **82**, 11.

[4] Oriel, G. H., and Barber, H. W., *Lancet*, 1930, II, 231.

[5] Pregl, F., *Arch. Physiol.*, 1899, **75**, 87.

[6] Munk, I., *Virchows Arch.*, 1877, **69**, 350.

based on the findings that strong alcohol does not dissolve certain interfering substances such as ergothioneine, proteose, the barium salt of oxyproteic acid, or barium urate but does dissolve barium thiocyanate a somewhat improved procedure has been employed as follows: An aliquot of urine generally 50 cc. is treated with a saturated aqueous solution of barium hydroxide as long as a precipitate forms. The mixture is filtered. The filtrate is brought to dryness on the water bath and is extracted with warm absolute alcohol and filtered. The procedure of extraction with alcohol and filtering is repeated. Then the alcohol is evaporated, the residue dissolved in 25 cc. of water and precipitated with silver nitrate. Then the Rupp-Schied-Thiel iodine procedure is applied.

Such a procedure has given a return of 97.2% for potassium thiocyanate in pure solution, a return of 95% for thiocyanate added to the urine and shows in urine amounts of thiocyanate very much smaller than the original Rupp-Schied-Thiel procedure applied to urine directly. Four normal urines tested for thiocyanate by this procedure gave values of 14.2 mg., 5.31 mg., 17.6 mg., and 25.5 mg. of HCNS in 24-hour samples as compared respectively with 101.3 mg., 66.2 mg., 116.1 mg., and 140.6 mg. HCNS in the original Rupp-Schied-Thiel procedure.

6685

Dissociation of Pneumococcus: A New Colony Variant.

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I. *A New Colony Variant of Pneumococcus.* R colonies of pneumococcus, suitably spaced on blood agar, are allowed to age for 6 to 7 days at 37° C. Under these conditions many of the colonies give rise to a variety of secondary growths including daughter colonies, peripheral excrescences and a peculiar form of localized, marginal outgrowth with a rough, fungoid appearance. These various forms of secondary growth have been observed in some detail and the rough, localized, marginal outgrowth represents an unusual phase of development. When transfers are made from such areas to fresh blood agar plates a new type of colony is produced. Its appearance varies considerably with age, but at all periods of growth is characteristically different from the parent colony. Young colonies (4-8 hours) present an extremely rough and irregular surface with delicate, filamentous edges. With age the surface is coarsely rough with an irregular fimbriated border. At 12-16 hours the surface is slightly convex, but after 24 hours the colonies become flattened. They continue to show an irregular margin and are approximately twice the diameter of the parent R colonies grown under similar conditions. With further ageing the colonies develop an extraordinary, spreading, filamentous type of marginal growth.

Stained smears of such colonies show an unusual morphological picture, varying with age, but quite distinctive at all periods of growth. The bacteria consist mostly of plump cocci and cocco-bacilli arranged in long chains. In younger colonies the organisms are almost exclusively Gram positive, but in the older ones there is considerable variation in staining properties. In addition to the plump coccal and cocco-bacillary forms, large numbers of granular elements of various sizes are present.

The stages in the development of the marginal outgrowth which produces the new type of colony have been carefully studied. The available evidence suggests that the transition to the new form is a gradual one. At first the organisms tend to grow in clusters suggestive of a staphylococcal arrangement. The next become pleomorphic and frequently elongated in an extreme, lanceolate manner. In the next stage they appear as pointed, diphtheroid-like elements arranged in a fashion suggesting broken twigs. Long, bizarre, rod forms may appear, which are partly Gram positive and partly Gram negative. Finally, the plump cocco-bacillary forms appear. Each of these morphological stages is represented by a characteristic colony type showing a gradual transition to the new form. The intermediate stages are moderately stable in both liquid and solid media; but, after a time, there appears to be a tendency toward reversion to the original parent form or a progression toward the new colony type.

When transferred to liquid media, colonies of the new type produce a sedimented type of growth resembling a tuft of cotton in the bottom of the tube. The supernatant is usually clear but there may be present loose, flocculent clumps in an otherwise limpid medium. Stained smears of such growths reveal a tangled mass of enormous chains and skeins of plump coccal forms. On solid media the new colony form appears quite constant and has continued to reproduce its own form for many transfers. In liquid media the new variant reproduces its own form for a number of transfers, but usually between the fourth and sixth subculture a change occurs. Diplococci of the usual morphology appear in the cultures, and colonies of 2 distinct types develop on plates streaked from such cultures: (1) those above described and (2) the original parent type. Further transfers in liquid media result in the appearance of an increasing number of cells of the original parent type until the entire culture appears to revert to its original form.

The morphological evidence suggests that the development of the new colony type is an indirect and not a direct product of the parent colony. On the other hand, reversion of the new colony type to the parent form appears to be sudden and abrupt. The organisms so far studied in the new variant form are bile-soluble, autolyze readily and possess relatively little virulence for white mice. Bacteria-producing colonies similar to the extremely rough variant above described and showing similar growth characteristics are frequently found in normal throats.

In a recent communication, Hadley[1] postulated the existence of this new variant form of pneumococcus and predicted its salient characteristics in a remarkable manner. The basis for Hadley's forecast and the place of the new colony variant in the phenomenon of bacterial dissociation will be considered in a subsequent communication.

6686

Effect of Sodium Chloride in Treatment of a Patient with Addison's Disease.

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It has been shown recently that the sodium content of the blood of patients suffering from Addison's disease is decreased[1] as it is in adrenalectomized cats,[2, 3] and that adrenalectomy in dogs results in a similar change, due to loss of sodium through the kidneys.[4] Soddu found that the symptoms of adrenal insufficiency in dogs "were relieved slightly by saline injections"[5] and many investigators, notably

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[1] Hadley, P., personal communication.

[1] Loeb, R. F., *Science*, 1932, **76**, 420.

[2] Marine, D., and Baumann, E. J., *Am. J. Physiol.*, 1927, **81**, 86.

[3] Zwemer, R. L., personal communication.

TABLE I.

Date	Total Base	Na	K	Ca	Cl	HCO_3	PO_4	SO_4	Serum Protein	Non-Protein Nitrogen	Glucose	Blood Pressure	Remarks
	m.eq. per l	m.eq. per l	m.eq. per l	m.eq. per l	m.eq. per l	m.eq. per l	m.eq. per l	m.eq. per l	m.eq. per l.	mg. per 100 cc.	gm. per l.	mm. Hg.	
7-19-32	136.3	123.5	5.3	5.3	88.6	21.8	2.6	2.3	15.1	39.0	0.73	85/55	Blood taken shortly after admission. Profound weakness and vomiting.
7-26-32	125.8	107.8	7.1	4.8	72.7	21.5	2.4		16.9	45.0	0.80	65/48	Critically ill. Almost *in extremis.*
8- 2-32	147.5	133.0	5.1	5.9	93.8	27.5	2.6		13.5	20.6	1.20	84/60	No more vomiting, sitting up. Treated with one dose of Eschatin and then NaCl.
11-14-32	150.5	139.9	4.6	5.5	107.3	24.3	2.2		14.4	20.0	1.16	112/74	Up and about at home, doing part of housework. Eats 7 gm. NaCl daily plus diet. Slight puffiness of eyelids.
1-24-33	139.5	126.8	5.7	5.6	92.3	21.2	2.5		16.6	35.0	0.91	86/60	After 7 dys. salt-poor diet weak and vomiting frequently. Confined to bed.
1-30-33	150.1	138.0	5.0	5.2	103.5	25.9	2.2		15.1	25.0		122/80	After no further treatment except NaCl added to diet and given by rectum. No vomiting. Much stronger.

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[4] Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J., in press.

[5] Britton, S. W., *Physiol. Reviews*, 1930, **10**, 617.

Marine and Baumann,[2] have shown that injections of salt solution prolong appreciably the life of adrenalectomized cats. Rogoff[6] has stated that "the intravenous administration of physiologic solutions of sodium chloride" becomes an "indispensable adjuvant in the treatment of Addison's disease with extracts representing the hormone of the adrenal cortex." This investigator attributes the action of NaCl solutions to the relief of "toxic accumulation" for which no evidence has been presented other than the accumulation of urea in the blood. In view of these facts, the responses of a patient with Addison's disease to the feeding and withdrawal of sodium chloride have been followed over a period of 6 months. This patient (P.H. 347860) presented the classical picture, complaining of progressive weakness, loss of appetite and weight and increasing pigmentation of the skin, nipples and buccal mucous membranes. Her blood pressure when first seen was 90/60. X-rays of the chest and abdomen were negative. There was a mild secondary anemia. Urine and stool examinations were negative.

The results of the various studies of the blood are shown in Table I. During the first week following admission, the patient's condition grew progressively worse, until the seventh day, when she became critically ill and her blood pressure fell to 65/48. Her complaints were profound exhaustion, slight dyspnoea and increasing nausea and recurrent vomiting. There was never any suppression of urine. The abnormalities present in the blood on the second morning after admission had increased enormously as her condition became more grave. Because of the decreased sodium concentration in the blood it was decided to try the effects of large doses of NaCl. (A single dose of 10 cc. of Eschatin was given intravenously at the onset of treatment, but this amount could hardly be adequate to influence materially the subsequent clinical course.) She was given about 9.5 gm. of NaCl a day by proctoclysis and 5.0 gm. daily by mouth in capsules. Within 4 days the clinical improvement was so striking that the rectal administration of salt was stopped, but the patient continued to take between 5 and 7 gm. of salt a day, in addition to a liberal amount in her diet. Table I shows the simultaneous improvement in the blood picture. The patient continued to gain in strength and was discharged on a regular diet, plus 1½ teaspoonsful of salt daily. Her blood pressure was at this time 102/70. After 6 weeks of this regime at home, the patient was able to do part of her housework and had vomited but once. Her blood pressure was 106/70 and she had gained 7 pounds. Three months after discharge, she had gained 4 pounds more and her blood pressure had risen to 112/74. There had been no more vomiting, but she noted slight puffiness under her eyes in the morning. Blood studies at this time (11-14-32) showed no abnormalities except that the chloride level had slightly surpassed the normal. Five months after discharge, while still eating 7 gm. of salt daily in addition to her diet, the patient reported that she was quite well. She had vomited once after strenuous Christmas shopping. She complained of some puffiness of her ankles at night and of her eyes in the morning. Her blood pressure was 122/80 and the urine was normal.

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[6] Rogoff, J. M., *J. Am. Med. Assn.*, 1932, **99**, 1309.

In view of the presence of slight edema, the patient was advised to discontinue NaCl administration and to take a salt-poor diet. One week later, she was admitted to the hospital with the story that after 2 days of this regime she became so weak that she was forced to go to bed. For the 5 days preceding admission, she became progressively weaker and vomited at frequent intervals. She had lost 7 pounds in one week, her blood pressure had dropped to 92/70 and by the next morning it was 86/60. Examination of the blood on this day (1-24-33) showed the striking drop in sodium and the other findings characteristic of adrenal insufficiency. The patient was given 7 gm. of NaCl by mouth daily and rectal taps of 200 cc. of physiological salt solution every 4 hours. No other therapeutic agents were used. After 3 days, her clinical condition had improved strikingly and the taps were discontinued. On the eighth day after admission her pressure was 122/80, she had gained 4 pounds and was discharged the next day feeling very well. The blood findings at this time (1-30-33) were once more essentially normal.

In a typical case of Addison's disease, the symptoms of severe adrenal insufficiency were relieved by treatment consisting of the administration of NaCl by mouth and by rectum. The patient remained in a state of comparatively good health for 5 months, during which time she ingested 7 gm. of salt in addition to her diet. Withdrawal of salt resulted in a recurrence of the symptoms of severe adrenal insufficiency requiring prompt hospitalization. This attack of adrenal insufficiency was also treated by the administration of sodium chloride, with the result that the patient was discharged symptom-free after 9 days. The correlation between the clinical condition and the changes in concentration of sodium in the blood is obvious.

This fact associated with the evidence that adrenalectomy in dogs causes a striking loss of sodium through the kidney[4] makes it reasonable to assume that the beneficial effects of NaCl upon the symptoms of adrenal insufficiency in this patient were due to the replacement of sodium lost from the body.

6687

Production of Extreme Pulmonary Compression and Cirrhosis.

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The purpose of the experiment was to determine how much a capsule of fibrous tissue surrounding a pulmonary lobe would compress the lobe by spontaneous contraction, especially when no adhesions existed between the lobe and its environs. The visceral pleura of the lobe was caused to produce the fibrous capsule by the envelopment of the lobe in a loosely fitting sac of foreign material.

Three dogs were anesthetized and operated upon aseptically. The right lower lobe was exposed through an intercostal incision and was wrapped completely in an envelope of thin rubber (2 dogs) or of surgical gauze (1 dog), care being taken that neither the lobe nor the vessels at the hilus were compressed appreciably by the sac, even when the lobe was fully expanded. The chest was then closed, without leaving pneumothorax. The dogs were allowed to survive and were examined roentgenographically from time to time. Sacrifice and autopsy were done 5 months after operation in one case with a rubber sac and 7 months after operation in the other cases.

All 3 dogs recovered from the operation and returned to vigorous health. One with a rubber sac developed a massive accumulation of exudate in the right pleural space and the others showed much smaller accumulations; but the exudate gradually disappeared to a large extent. At autopsy in the cases with rubber sacs, the right lower lobe was found to be still contained within the envelope, which was now much crumpled, and to be reduced to about the size and shape of the 2 terminal segments of a man's finger, smooth and firm. Section showed that the lobe was covered with a heavy fibrous capsule and was composed of grey, densely packed fibrous tissue in which a few completely collapsed bronchi were discernible. There was no excess of pleural fluid. In the case with the gauze sac, the right lower lobe was surrounded by a fibrous capsule as thick as, or thicker than, that in the other dogs. The capsule encompassed and permeated the gauze and attached the lobe firmly to the ribs, diaphragm and other surrounding structures. The lobe was uniformly air-containing and about the same size as the other lobes when the lungs were removed and allowed to deflate. On section, its parenchyma was found to be congested but otherwise normal in gross appearance.

Histological examination of the lobes encased in rubber showed that the visceral pleura was transformed into a thick layer of scar tissue and the parenchyma was crossed in many directions by an irregular network of wide fibro-hyaline trabeculae. Between the trabeculae lay tightly compressed bronchi and connective tissue but no alveoli or bronchioles. The main bronchus was so greatly compressed that the cartilages were bent, broken and imbricated, although the mucosa was intact. Nowhere was there sign of inflammation or necrosis. The blood vessels seemed patent. The lobe covered with gauze looked quite different except for the presence of the fibrous capsule. The structures were all present, the alveoli were partially air-containing, a few areas presented leukocytic or erythrocytic infiltration, and cellular debris occupied some of the bronchioles.

Thus, all 3 right lower lobes were compressed to some extent. The one wrapped in gauze was reduced in size but not to the extent of airlessness; while the lobes covered with rubber were compressed not only to airlessness but much more. That is, the latter were very much smaller than they would have been if atelectatic and uncompressed. This implies that much of the tissues of the compressed lobe had been caused to disappear. The reason for the difference between the effects of the 2 kinds of sac seems to be that, in the dog with a gauze sac, fibroblasts penetrated the gauze, bound the lobe to its environs, and permitted the ribs and the other more or less rigid parts surrounding the lobe to resist the contraction of the fibrous capsule to a considerable extent; whereas, in the other dogs, the fibroblasts were unable to penetrate the rubber so that the visceral capsule was left perfectly free to contract. The elasticity of the rubber could not have caused the compression, since the sac was loose even at the start.

The causes for the development of cirrhosis and for the disappearance of so much of the parenchyma need elucidation.

6688

Concentration of Congo-Red in the Blood after Absorption from the Pneumonic Lung.

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The pulmonary circulation undergoes marked alterations in pneumonia, (1) to a condition of hyperemia and (2) to that of ischemia. Upon the state of efficiency of the circulation in the infected tissues must depend largely the rate of absorption of the toxic products of the infection, and upon the latter must depend the severity of the toxic symptoms. These experiments represent the initial phase in a study of the absorption rate as a basis for a better understanding of the symptomatology of pneumonia. Congo red was the substance injected into the lung for testing the absorption of soluble products, because it is identifiable and measurable in the blood, and, in the normal individual at least, is eliminated from the blood (by the liver) at a slow and uniform rate as compared with other non-toxic dyes. The only diseases known[1] to alter appreciably the rate of elimination of congo red are amaloidosis, which retards it, and nephrosis and cirrhosis, which sometimes accelerate it, but these are not found in dogs.

Pneumonia was produced in 24 dogs by the method of Terrell, Robertson and Coggeshall,[2] with the modifications that the pneumococci (type 1) were suspended in saline rather than in the more viscid medium, to allow them to reach the peripheral airways more easily, and that the inoculation was made bronchoscopically. Morphine, atropine and ether were given previously, and 3 cc. of a thin inoculum was injected into one bronchial branch of the right lower lobe. After an interval of 2, 4, 24, or 48 hours, the animals were anesthetized again, a sample of blood was obtained, congo red (1 cc. of 1.25% solution per kg. body weight) was injected very slowly into the same bronchus as was used for the inoculation, and samples of blood were collected at 10-minute intervals thereafter for nearly 2 hours. Each sample was drawn into isotonic solution of sodium oxalate (9 parts of blood to 1 part of solution), the mixture was fractionated by centrifuging, and the supernatant fluid was analyzed colorimetrically. Immediately after the last sampling the animals were killed and autopsied, with special attention to the nature of the lesion and the distribution of the dye still remaining in the lungs. Six dogs were used as controls, treated in the same way except that the inoculation was omitted.

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[1] Bennhold, H., *Arch. klin. Med.*, 1923, **142**, 32; *Klin. Wchnschr.*, 1924, **38**, 1711.

[2] Terrell, E., and Robertson, O., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 973. Terrell, E., Robertson, O., and Coggeshall, L., *J. Clin. Invest.*, 1931, **10**, 659.

The control dogs began to show a trace of dye in the blood at 10 to 20 minutes after injection and a maximal concentration of dye of 6.25 to 11.25 parts per million at 40 to 60 minutes after injection. The dogs examined 2 hours after inoculation had an appearance-time of 10 to 30 minutes and a maximal concentration of 3.75 to 11.25 parts at 40 to 70 minutes. Those examined 4 hours after inoculation had an appearance-time of 10 to 40 minutes and a maximal concentration of 3.75 to 11.25 parts at 60 to 80 minutes. Those 24 hours after inoculation had an appearance-time of 20 to 50 minutes and a maximal concentration of 0 to 6.25 parts at 50 to 70 minutes. Three of this group presented no measurable amounts of dye at any time. Finally, the dogs examined 48 hours after inoculation had an appearance-time of 30 to 50 minutes and a maximal concentration of 0 to 6.25 parts at 60 minutes. Four of this group showed no measurable amounts of dye. Fig. 1, representing the simple averages for the 5 groups, demonstrates the outstanding characteristics, namely, the gradual rise and fall of the concentration of dye in the blood in all cases, and the tendency for a marked delay in the time of appearance and a great reduction in the maximal concentration in the cases with pneumonia, already distinctly in evidence 2 hours after inoculation. The lesions appeared at autopsy to be the same as those described by the authors of the method.[2] Those at 2 or 4 hours after inoculation were characterized by intense swelling, edema, congestion and hemorrhage, and those at 24 to 48 hours, by red-hepatization. There was considerable variation

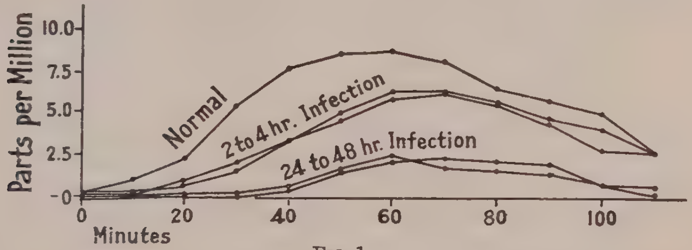

FIG. 1.

Curves representing the average behavior of the dye-concentration curves for each of the 5 series of dogs.

in the extent and advancement of the lesions in the different individuals at each period after inoculation and, on the whole, this was proportional to the degree of the abnormalities in the dye-concentration curves. The dye was confined almost wholly to the right lower lobe. In the cases with pneumonia, the dye was in the tissues of the lesion, and also in the tissues immediately adjacent when the lesion did not occupy the entire lobe. That the dye penetrated well into the lesion was evidenced by its being visible plainly on both the pleural and cut surfaces of those tissues. The bronchi in the lesion large enough for gross examination were found to be patent but intensely stained.

The abnormalities of the concentration of dye in the blood of the dogs with pneumonia may have been due to one or both of 2 causes: (1) a delay in the onset, and a diminution in the rate, of absorption of the dye from the diseased lung, and (2) an increase in the promptness and rate of elimination of the dye from the blood. If the latter factor enters at all, it is probably of minor consequence, judging from what is known of the regularity of the process and elimination of congo red in disease in man; but to judge the rate of absorption from the lung with certainty, it will be necessary to determine whether or not the rate of elimination in dogs is affected by pneumonia after injection of the dye into the blood-stream. This is the next phase of the study.

The extremely early period of pneumonia at which marked abnormalities of dye absorption rate were seen is particularly noteworthy. Evidently the efficiency of the circulation in the infected tissues is greatly reduced at the stage of congestion in pneumonia, although not as greatly as at the stage of consolidation.

6689

Electromyographic Studies of Gastro-intestinal Tract. II. Is There a Gradient of Electrical Potential Along the Small Intestine?

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In 1922, Alvarez[1] mentioned briefly studies that led him to suspect that at least in certain animals there might be a gradient of electrical potential along the small bowel with the duodenum negative to the parts distal to it. Unfortunately the technic was not entirely satisfactory and at times the gradient was not evident, so the subject was left open for further research.

In the experiments here reported an effort has been made to get definite information with a more reliable technic. Rabbits, anesthetized with urethane and with the abdomen open, were placed in a wooden tank filled with physiologic salt solution at 37°C. The thermostat and the heating element were enclosed in glass. Under these conditions there was no obvious source of electrical potential that could set up stray currents. To make sure of this it was determined experimentally that the bath was everywhere at equal potential.

Some mineral oil was floated over the saline solution and the loops of intestine being studied were allowed to float up into the non-conducting layer. In order to avoid possible changes in potential due to the presence of material in the lumen of the bowel, the gut was washed through with physiologic salt solution. A non-polarizable silver-silver-chloride electrode was left attached to the duodenum near the pylorus and differences in potential at various points along the bowel were measured with the help of another electrode of the same type. The differences were measured with the calibrated control board of an electrocardiographic apparatus. When so used it becomes a sensitive potentiometer with the string galvanometer as the null instrument. Readings were made to the nearest millivolt.

Observations were made on 10 rabbits. First measurements were made at about 10 successive points on the intact small bowel. The gut was then excised, and its lumen was again washed through with saline solution, similar measurements were made at the same points, identified by markers. In Fig. 1 are presented the results of the

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[1] Alvarez, W. C., and Mahoney, Lucille J., *Am. J. Physiol.*, 1922, **58**, 475.

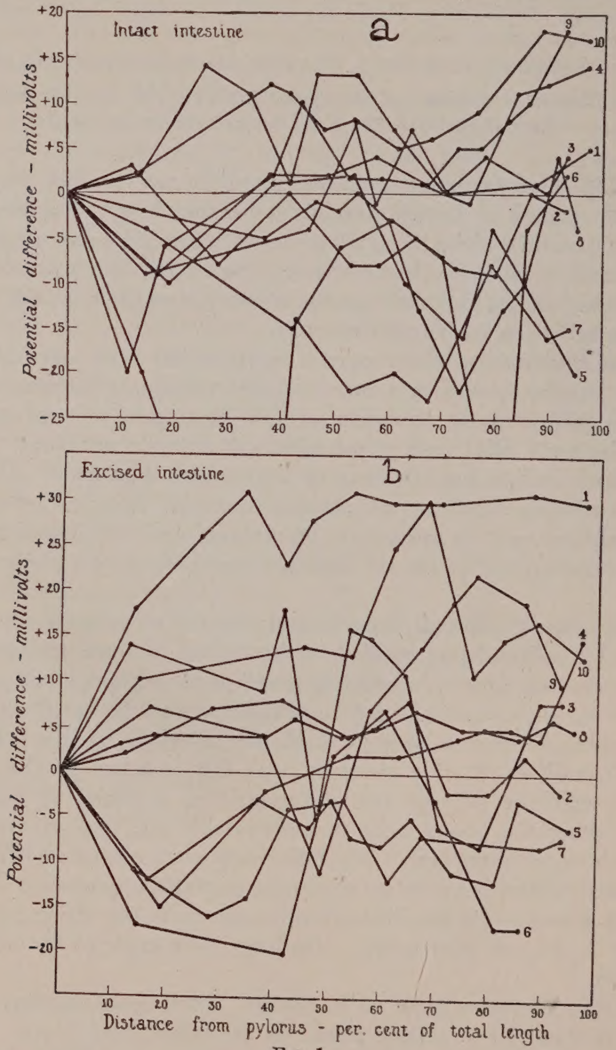

FIG. 1.

Potential differences measured with reference to the pylorus for different points along the intestine. The distance of the point, measured from the pylorus is expressed in % of the total length from pylorus to tip of appendix; *a*, the intestine intact in the animal, each numbered curve is for an individual animal; *b*, the intestine excised, the numbers correspond to those in *a*.

individual observations, and in Fig. 2, the averages. It will be seen that, both in the intact and the excised bowel, there was no uniform trend of potential along the bowel.

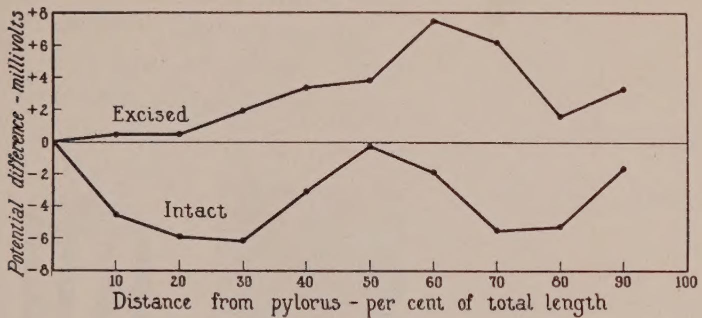

FIG. 2.

The averages for the 10 experimental animals shown in Fig. 1.

Considering the marked individual variability it was rather surprising to find a high degree of parallelism in the mean values obtained with intact and excised bowel. As one might anticipate from Fig. 2, the correlation between the potential values obtained from the same point of the intact and excised intestine was significantly high. The coefficient for the entire series of observations was +0.55 ±0.05.

It is impossible to say what significance these observations have. All we can say is that whatever the factors are which determine the potential difference between any 2 parts of the bowel they tend to remain more or less constant at least for a short time after the removal of gut from the animal.

Summary. There is no constant gradient in electrical potential along the small bowel of the rabbit. The values that are found tend to persist for a while after excision of the bowel.